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STUDIES ON THE INTERACTION OF A TEMPERATURE SENSITIVE MUTANT OF MEASLES VIRUS WITH PERIPHERAL BLOOD MONONUCLEAR CELLS

by

SOOPAYAH VYDELINGUM ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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OF Ph.D. IN MEDICAL SCIENCES (VIROLOGY)

DEPARTMENT OF MEDICAL MICROBIOLOGY AND
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....

Supervisor

....

Bhagirath Singh

Glen Armstrong

R. Marusyk

Date: Nov. 26, 1988

To DIVA,

My brother, my father, my friend

Abstract

The interaction of measles virus (MV) with peripheral blood mononuclear cells (PBMC) has been studied using a temperature sensitive (ts) mutant isolated in Vero cells. The virus retained the ts characteristics in PBMC at 37°C. At the restrictive temperature in Vero cells, an early block prior to transcription of the viral genome was observed. In PBMC, on the other hand, a late block in viral replication was responsible for the ts characteristics as viral mRNA and proteins were detected at the restrictive temperature. Under such conditions, the virus abrogated the proliferation of stimulated PBMC.

Monocytes were found to play an important role in the inhibitory effect of the virus. T cells, on the other hand, did not play a significant role in MV ts38-induced inhibition of PBMC proliferation. The importance of monocytes could not be explained by preferential replication of the virus in these cells as more infectious virus was released from T cells. However, there was no significant difference in the amount of viral protein synthesized in MV ts38-infected T cells compared to MV ts38-infected monocytes. Maturation of monocytes *in vitro* had no effect on the inhibitory effect of the virus. No significant decrease in viable cell number was observed in MV ts38-infected PBMC as compared to uninfected PBMC, indicating that the cytopathic effect of the virus did not contribute to the inhibitory effect. There was no decrease in interleukin-2 level in MV ts38-infected cells. However, significant amounts of interferon α (IFNα) was released. Partial but significant reversal of inhibition was obtained with anti-IFNα, indicating that IFNα was one of the mediators of the inhibitory effect of the virus. Inactivated virus did not interfere with PBMC proliferation.

Supernatant solutions (SN), obtained from MV ts38-infected PBMC blocked the proliferation of continuous cell lines in vitro. Anti-IFNα partially reversed the inhibitory effect. Removal of viral proteins from the SN did not decrease the inhibitory effect of the SN on continuous cell lines. Monocytes were found to be responsible for the secretion of the inhibitory factor(s).

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I would like to express my deepest gratitude to my supervisor, Professor Aimo Salmi, without whom this study would not have been possible. His wide knowledge of virology and his readiness to listen and help brought hope in time of despair. I would also like to express my gratitude to Professor Raymond Marusyk for his expert guidance and support during the course of this study. I am grateful to Professor D. L. J. Tyrrell, Chairman of the department whose support was invaluable for the completion of this work. I would like to thank Dr. R. Whitehouse for revision of the text. I would also like to thank L. Chui, and [illegible] K. Suryanarayana for their expert technical help; R. [illegible]ne and S. Vinh for their help in photography. A special thanks to my friends Anona Lukawiecki, Catherine Moran and Peter Pieroni who were there when I needed their support. I am deeply grateful to all members of my family for their support and understanding over the years.

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List of Abbreviations

A - Actin

5-BUdR - 5-bromodeoxyuridine

C - Non-structural protein of MV

CDV - Canine distemper virus

DNA - Deoxyribonucleic acid

EIA - Enzyme immunoassay

F - Fusion protein of MV

FITC - Fluorescein isothiocyanate

FUdR - 5-fluorodeoxyuridine

H - Hemagglutinin protein of MV

HIV - Human immunodeficiency virus

HLA - Human leucocyte antigen

HSV - Herpes simplex virus

IU - International unit

IFN - Interferon

IL-2 - Interleukin-2

L - Large protein of MV

LCMV - Lymphocytic choriomeningitis virus

LDV - Lactic dehydrogenase virus

LPS - Lipopolysaccharides

M - Matrix protein of MV

m.o.i. - Multiplicity of infection

mRNA - messenger ribonucleic acid

MV	- Measles virus
N	- Nucleocapsid protein of MV
NU	- Neutralizing unit
P	- Phosphoprotein of MV
PAGE	- Polyacrylamide gel electrophoresis
PBMC	- Peripheral blood mononuclear cells
pfu	- Plaque forming unit
PHA	- Phytohemagglutinin
RNA	- Ribonucleic acid
RSV	- Respiratory syncytial virus
SN	- Supernatant solutions
SSPE	- Subacute sclerosing panencephalitis
ts	- Temperature sensitive
VSV	- Vesicular stomatitis virus

INTRODUCTION

A. General

The interaction of viruses with peripheral blood mononuclear cells (PBMC) has been under scrutiny for several decades, more so presently in light of the fatal consequences of human immunodeficiency virus (HIV) infections. Many viruses including rubella, measles, mumps and HIV replicate in PBMC (Mims, 1986) and in so doing, evade or subvert the immune system.

The consequences of virus replication in PBMC vary depending on the virus and cell involved. HIV infects CD4 positive cells and causes a permanent immunosuppression as a result of the gradual depletion of CD4 positive T cells. Lactic dehydrogenase virus (LDV), on the other hand, replicates in macrophages or dendritic cells and as a result, antigen handling or presentation is perturbed. Measles virus (MV), however, infects T cells, B cells and monocytes/macrophages and in so doing, induces a severe but transient immunosuppresion. Both HIV and MV can persist in the body, leading to neurological disorders, years later.

The infection and replication of viruses in PBMC can be viewed as a viral strategy for survival. It entails spread throughout the body, enabling the virus to reach immunologically privileged sites. Persistency ensures the presence of the virus within the host and if viruses are excreted to the outside, within the host species as well.

Studies on virus interactions with PBMC are essential in understanding how viruses can subvert or evade the immune system. Such studies will also help understand the possible ways by which viruses can induce persistency and survive not only within the host but also within the host species.

B. Measles virus (MV)

1. Historical perspective

Measles virus literature dates back to the 10th century, when an Arabian physician, Rhazes, first described the disease (Wilson, 1962). However, no clear distinction was made between measles and smallpox until the beginning of the seventeenth century, when each was listed separately in the annual bill of mortality prepared by the parish clerks of London (Wilson, 1962).

Evidence suggesting that a virus might be the causative agent of measles was first reported in 1905, when it was shown that measles developed in susceptible volunteers following inoculation of bacteria-free blood obtained from diseased individuals (Hektoen, 1905). It was later shown to be transmitted from diseased to healthy monkeys via throat washings (Anderson *et al.*, 1911). Starting in the 1930s, several reports dealing with the propagation of the virus in chick embryos and chick tissue cultures were published (Rake *et al.*, 1939, 1941), culminating with the isolation of the virus in 1954 (Enders *et al.*, 1954) from blood of a patient suffering from measles. The virus was propagated *in vitro* using monkey and human cells, resulting in the first description of the pathologic changes induced by the virus.

2. Classification

The family Paramyxoviridae comprises three genera: Paramyxovirus, Morbillivirus and Pneumovirus. All members within the family are enveloped, pleomorphic viruses. Each has an internal helical nucleocapsid and a linear, single-stranded, negative sense RNA that is complementary to the mRNAs. The viruses replicate in the cytoplasm. The paramyxovirus genus contains the Sendai virus of mice, mumps virus, the avian paramyxoviruses and four parainfluenza virus types. The viruses possess both hemagglutinating and neuraminidase activity. The morbillivirus genus consists of measles virus (MV), canine distemper virus (CDV), rinderpest virus of cattle and peste des petits ruminants virus of goats and sheep. These viruses lack detectable neuraminidase activity, hence their classification in a separate genus from the paramyxoviruses (Kingsbury *et al.*, 1978). The pneumovirus group contains the respiratory syncytial viruses (RSV) of humans and cattle and the pneumovirus of mice. The pneumovirus differs from the two other groups in a lack of hemagglutination activity and the size of the nucleocapsids (Norrby *et al.*, 1970).

3. Physical characteristics of the virion

Measles virions are pleomorphic and range in size from 100 to 300 nm in diameter. Larger virions are occasionally observed (Nakai ~~*et al.*~~, 1969; Lund *et al.*, 1984). The virion consists of an internal nucleocapsid surrounded by an envelope (Morgan *et al.*, 1977; Fraser *et al.*, 1978; Norrby, 1985). The envelope consists of short projections, 9 to 15 nm in length, and a lipid membrane of cellular origin which is 5 to 8 nm wide (Waterson *et al.*, 1961; Waterson, 1965; Morgan *et al.*, 1977). The short projections observed on the virions are the glycoproteins F and H, responsible for the fusion and hemagglutinating activities, respectively (Hardwick *et al.*, 1978). Inside the viral membrane is a helical nucleocapsid of between 17 to 21 nm in diameter and ranging in length from 1100 to 1600 nm. It has a central core of around 5 nm in diameter which harbors the viral RNA (Nakai *et al.*, 1969; Lund *et al.*, 1984). MV has a buoyant density between 1.29 to 1.30 g/cm^3 as determined by equilibrium centrifugation in cesium chloride gradients (Bussell, 1974; Robbins *et al.*, 1980).

4. Genome organization

The presence of RNA in MV was first demonstrated in 1964, when it was shown that 5-bromodeoxyuridine (5-BUdR), a thymidine analogue, failed to inhibit MV replication (St. Geme, 1964). The genome consists of a single stranded RNA of negative polarity, which has a sedimentation rate of 50 to 52S and a molecular weight of 4.5 to 5.2 x 10^6, as determined by electrophoretic analysis and electron microscopic studies (Kingsbury *et al.*, 1978; Baczko *et al.*, 1983; Lund *et al.*, 1984; Udem *et al.*, 1984). The genomic RNA is surrounded by nucleocapsid protein subunits which together constitute the nucleocapsid. Purified MV nucleocapsids form a sharp band in CsCl gradients at a density of 1.33 g/mL (Andzhaparidze *et al.*, 1987). Two types of nucleocapsids have been observed in MV infected cells; fuzzy types, found in the cytoplasm of infected cells and smooth ones seen mainly in the nucleus (ter Meulen *et al.*, 1973; Dubois-Dalcq *et al.*, 1974). The MV genome is about 15,000 nucleotides in size and contains seven genes, six of which code for major structural proteins. The gene order, starting from the 3' end is as follows: 3'-nucleoprotein (N) - [phospho (P) + non-structural (C)] - matrix (M) - fusion (F) - hemagglutinin (H) - large (L)-

5'(Richardson *et al.*, 1985; Dowling *et al.*, 1986; Crowley *et al.*, 1987). The first RNA transcribed from the 3' end is a leader sequence of 57 nucleotides (Billeter *et al.*, 1984; Crowley *et al.*, 1988). This is followed by the N gene located at the 3' end which is transcribed into a 1,750 nucleotide long mRNA, starting 109 nucleotides from the 3' end of the genome, coding for the N protein. The P+C gene is composed of 1,657 nucleotides and specifies a 507 amino acid protein, the phosphoprotein. The gene also contains a second reading frame, starting 22 nucleotides from the first ATG triplet, specifying a 186 amino acid protein, a non-structural protein designated C (Bellini *et al.*, 1985). The entire M gene is 1,463 nucleotides in length, coding for a protein of 335 amino acids. It has a noncoding region of 425 nucleotides, located at the 5' end of the gene (Bellini *et al.*, 1986). The F gene is 2,477 nucleotides long and is transcribed into a mRNA of 2,384 nucleotides long which codes for a polypeptide with 553 amino acids (Richardson *et al.*, 1986; Buckland *et al.*, 1987). The hemagglutinin gene is 1,960 nucleotides in length and specifies a protein of 617 amino acids (Alkhatib *et al.*, 1986). The L gene, located at the 5' end of the genome, is slightly more than 6,350 nucleotides long coding for a protein of 2,183 amino acids (Crowley *et al.*, 1987).

5. Viral proteins - structure and function

MV consists of six structural proteins and cellular actin. One non-structural protein, C, is present in infected cells. N, P and L are located internally in the virion. F and H are transmembranous proteins, and together with M, which lines the inside of the viral envelope, form the viral proteins of the envelope.

The nucleocapsid(N) protein is the major protein in the virion as well as in infected cells, and as the name implies, is the main component of the nucleocapsid. It has a molecular weight of 60,000 as determined by SDS-polyacrylamide gel electrophoresis. The mobility of the N protein varies slightly from strain to strain (Mountcastle *et al.*, 1977; Hall *et al.*, 1979). The variation in mobility has been attributed to the degree of phosphorylation of the protein (Wechsler *et al.*, 1978; Vainionpää, 1979). Serine and threonine are the two phosphorylated amino acids within the protein, serine being the major phosphorylated amino acid and threonine being phosphorylated to a lesser amount (Robbins *et al.*,1979).

Two other proteins, namely, large(L) and phospho(P) proteins, are also part of the nucleocapsid, but present in smaller quantities than N. The P protein is a phosphoprotein of molecular weight 70,000, with serine as the major phosphorylated amino acid. It has been isolated from nucleocapsids, derived from both infected cells and virions (Robbins *et al.*, 1979; Rima *et al.*, 1983; Bellini *et al.*, 1985). Although its function has never been determined, the P protein is considered to be part of the transcriptase complex by analogy to the paramyxoviruses (Kingsbury *et al.*,1978). The MV P protein contains a second overlapping reading frame, specifying a protein of 21,000 molecular weight, designated C (Bellini *et al.*, 1985). It was first described as an 18 to 20,000 molecular weight protein, present in MV-infected cells but not in purified virions (Vainionpää *et al.*, 1978). It has been shown by immunofluorescence that the C protein in both acute and persistent measles infections co-localizes with nucleocapsids. The co-localization has been observed in both the nucleus and cytoplasm suggesting that C may play a role in nucleocapsid assembly or function (Bellini *et al.*, 1985).

The L protein is the largest protein of MV. It has been identified in purified virions and in the cytoplasm and nuclei of infected cells (Tyrrell *et al.*, 1978; Graves, 1981). It is a minor component of the virus with an estimated molecular weight of over 200,000. Its function is unknown but is thought to be part of the transcription and/or replication of MV together with P and the nucleocapsid protein (Stallcup et al., 1979) as described in paramyxoviruses (Kingsbury, 1985). Comparative studies show that MV L protein is highly conserved within the family Paramyxoviridae. Homology between the L protein of MV and vesicular stomatitis virus (VSV), a member of the family Rhabdoviridae, is restricted to charged and hydrophobic amino acids. These facts point to a similar function and common ancestral origin (Blumberg *et al.*, 1988).

Surrounding the nucleocapsid, giving it a fuzzy appearance in the cytoplasm, is the matrix (M) protein (Brown *et al.*, 1987), the smallest structural protein of the virus. M is a basic protein with a molecular weight of 37,000 (Mountcastle *et al.*, 1977; Hardwick *et al.*, 1978; Tyrrell *et al.*, 1978). The M protein of MV shares 76% homology with the M protein of CDV, and 35% similarity with that of Sendai virus. The combination of "...nonpolar, hydrophobic and β-sheets characteristic of the homologous region" within the different M proteins suggests that the protein may interact with the envelope and does not span the membrane (Bellini *et al.*, 1986). Strong association between the M protein and the nucleocapsid has been found in the cytoplasm but not in the nucleus (Stallcup *et al.*, 1979; Tyrrell *et al.*, 1980; Brown *et al.*, 1987). The presence of the M protein has also been detected on the inner surface of cytoplasmic membranes of MV infected cells and virion envelopes, suggesting a role in virus assembly, maturation and budding (Brown *et al.*, 1987).

The fusion and the hemagglutinating proteins are the only glycoproteins of the virion. Both proteins span the viral membrane protruding on the outside, making them immunologically important viral proteins. H is the larger glycoprotein, with a molecular weight of 79 to 80,000 (Mountcastle *et al.*, 1977; Tyrrell *et al.*, 1978; Vainionpää *et al.*, 1978) that mediates binding of the virus to host cells and erythrocytes (Krah *et al.*, 1988). It possess hemagglutinating but not neuraminidase activity. Carbohydrates constitute 12% of the total mass of hemagglutinin, a dimeric molecule linked by a disulphide bond (Hardwick *et al.*, 1978; Lund *et al.*, 1981). The only major hydrophobic region within the deduced amino acid sequence of the H protein, which is of sufficient length to anchor the protein in membranes, lies between amino acid residues 35 and 58 near the amino terminus of the molecule (Alkhatib *et al.*, 1986). Similar findings have been obtained with Sendai virus (Blumberg *et al.*, 1985).

The smaller glycoprotein, designated F, is synthesized as an uncleaved precursor, F_0 of 60,000 to 62,000 molecular weight (Graves *et al.*, 1978; Vainionpää *et al.*, 1978), which is converted to its biologically active form by proteolysis to form F_1 and F_2 that are held together by a disulphide bridge. F_1 is a 40,000 to 41,000 nonglycosylated protein and the F_2 portion is a 20,000 to 25,000 glycopeptide (Hardwick *et al.*, 1978; Tyrrell *et al.*, 1978; Vainionpää *et al.*, 1978). The F protein is responsible for cell fusion activity, which is located at the N-terminal region of the F_1 polypeptide of the virus. Comparative studies of the gene sequence of F from two MV strains show that the gene is highly conserved as no amino acid differences were observed (Buckland *et al.*, 1987). The overall homology of MV F protein with those of other paramyxoviruses is as follows: 25.3% with SV5, 26.5% with Sendai virus, 24.2% with parainfluenza 3 virus and 24.0% with NDV (Buckland *et al.*, 1987). The greatest homology (between 64 to 78%) was found between residues 112 to 140, which defines the hydrophobic region responsible for cell fusion. The region of least homology between the F protein of the paramyxoviruses is at the ends, i.e, the signal peptide, the transmembrane region and the cytoplasmic residues (Buckland *et al.*, 1987).

The F protein of MV mediates the entry of viral nucleocapsid from the attached virus into the cell, thus initiating infection through the fusion of viral envelopes and cellular membranes. The F protein is required for the spread of virus infection, via the transfer of viral genetic material from one cell to an adjacent one, through fusion of the cell membranes. The protein is also required for cell death as well as syncytium or multinucleated cell formation, giving it an essential role in pathogenicity. (Richardson *et al.*, 1986; Sato *et al.*, 1988).

A cellular protein, actin, has also been detected in the virion. Actin is considered part of the virus and believed to play an essential role in assembly and budding of the virus (Tyrrell *et al.*, 1978; Bohn *et al.*, 1986).

6. Replication

The first step in MV replication is adsorption, a prerequisite for infection. Adsorption occurs following the recognition of a cell surface receptor by the H protein of MV. Though the cell surface component for MV attachment has not been fully characterized, certain facts are known: sialic acid residues are not required for efficient binding of MV to cells (Howe, 1979), and the binding sites on both tissue culture cells and erythrocytes has a glycoprotein component (Krah *et al.*, 1988).

Following adsorption, the F protein mediates the fusion of the viral membrane to the cellular membrane, allowing the nucleocapsid to enter the cytoplasm. The genome is organised as a single transcriptional unit with a promoter site located at the 3' end. It serves as a template for the synthesis of viral mRNAs, which are capped and methylated at the 5' end and polyadenylated at the 3' end (Udem *et al.*, 1984). This process, known as primary transcription, is carried out by a structural RNA-dependent RNA polymerase (Siefried *et al.*, 1978; Ray *et al.*, 1987) and is independent of protein synthesis. Following the translation of the mRNAs, replication starts under the direction of the newly synthesized proteins. Plus (+) strand RNAs of genomic size are synthesized from the genomic parental RNA and serve as templates for the synthesis of genomic (-) strand RNA. The plus strand RNA that is a part of the replicative intermediate is different from the plus strand mRNA in size, i.e, it is of genomic size while mRNA is gene unit length. In MV-infected cells, six distinct species of monocistronic mRNAs can be detected together with six different readthrough RNAs (Hall *et al.*, 1978; Dowling *et al.*, 1986; Rima *et al.*, 1986; Yoshikawa *et al.*, 1986)

Viral assembly occurs in the cytoplasm following the synthesis of viral proteins. Viral glycoproteins, synthesized in an infected cell, are co-translationally inserted into the endoplasmic reticulum and transported to the cell surface via the Golgi apparatus, while undergoing modifications by the cellular machinery. Both the H and F proteins are glycosylated following insertion into the endoplasmic reticulum. Two oligosaccharide chains are N-linked to the F_2 domains of the F protein (Richardson *et al.*, 1986; Buckland *et al.*, 1987). Processing of the carbohydrate chains convert them to complex type sugars. Proteolytic cleavage of the F protein occurs following glycosylation and transport to the cell surface. Unglycosylated F and H are not transported to the cell surface, probably due to lack of proper configuration. Proteolytic cleavage of F is initiated intracellularly and continues to proceed extracellularly (Graves *et al.*, 1978; Sato *et al.*, 1988). The H glycoprotein is synthesized in an immature form and is processed to a mature form, by the conversion of its high mannose carbohydrate to a complex type one (Anttonen *et al.*, 1980; Kohama *et al.*, 1986).

Following synthesis, the glycoproteins are inserted into the cell membrane. The M protein is then associated with the inner surface of the lipid bilayer, interacting with the cytoplasmic part of the glycoproteins already in place. The M protein also serves as a recognition site for the proper alignment of the nucleocapsid beneath areas of the cell membrane that contain viral envelope proteins (Choppin *et al.*, 1981; Dubois-Dalcq *et al.*, 1984). The entire process ends with the budding of infectious particles. Actin is involved in the release of infectious virus from infected cells (Tyrrell *et al.*, 1978; Tyrrell *et al.*, 1979). The M protein serves as a recognition site for actin (Giuffre *et al.*, 1982), but the way actin associates with viral structures at the plasma membrane and its role in the process of budding are still uncertain (Tyrrell *et al.*, 1978; Bohn *et al.*, 1983; Bohn *et al.*, 1986).

C. Measles - a disease of childhood

1. Disease process[1]

MV is responsible for the common disease of early childhood, measles. It is highly infectious and has an incubation period of 10 to 12 days (Kempe *et al.*, 1965; Norrby, 1985). Infection is initiated in the upper respiratory tract of susceptible individuals, where local viral replication occurs, ensuring limited spread of the virus. This is followed by a primary viremia that ensures the distribution of the virus to distant sites, where the virus replicates actively in a wide variety of cells and tissues (Burnet, 1968; Katz, 1982; Moench *et al.*, 1988), leading to secondary viremia and widespread dissemination of the virus. Following the secondary viremia, virus can be detected throughout the body. At that stage of the infection the patient is highly infectious until a few days after the onset of rash. Viral replication decreases soon after and infectious virus can be isolated from the urine only. The rash disappears within a few days, followed by recovery which may be interrupted by secondary illnesses.

2. Complications

A wide variety of complications may arise during MV infections. These are mainly due to bacterial infections or respiratory tract disorders (Morgan *et al.*, 1977; Katz, 1982). Airway obstruction may be so severe that tracheostomy is required. Giant cell pneumonia, a usually fatal complication characterised by the presence of multinucleated giant cells in the respiratory tract, can occur in immunocompromised patients (Burnet, 1968; Katz, 1982). Secondary bacterial infections are mainly due to the invasion of the respiratory tract by pyogenic bacteria (Morgan *et al.*, 1977; Katz, 1982). Other complications, the most common of which is encephalitis, involve the central nervous system. Approximately 0.1% of measles infections lead to encephalitis (Katz, 1982).

Different types of MV-induced encephalitis have been described (Norrby, 1985). The most common is post infectious encephalitis appearing a short time after the rash. No virus is detected, implying an autoimmune reaction (Moench *et al.*, 1988). Acute measles encephalitis which occurs as a result of non-restricted replication of the virus in immune deficient hosts is another form of MV-induced encephalitis. MV infection is also responsible for a third type of encephalitis known as subacute sclerosing panencephalitis

(SSPE). It occurs at a frequency of one case per million acute measles virus infections, primarily in children and young adults with an average interval of 6 to 8 years between acute measles and SSPE (ter Meulen *et al.*, 1984). It has been attributed to the progressive dissemination of a defective virus (Norrby, 1985).

3. Epidemiology

Measles virus has a worldwide distribution. In nature, it infects only man and monkeys which are in contact with man. It has no known animal reservoir.

With the introduction of measles vaccines, the case-fatality ratio for children under three years of age has dropped from 10% to less than than 1 in 1000 in the industrialized world (Aaby *et al.*, 1986). However, in developing countries, measles still kills two million young children every year (Walsh, 1983; Marusyk, 1984). The most commonly cited factors to explain the high mortality in developing countries have been nutritional status, young age at the time of infection, lack of adequate care and genetic susceptibility (Coovadia *et al.*, 1981; Walsh, 1983; William *et al.*, 1983; Aaby *et al.*, 1988). In malnourished children, the rash becomes dark-red purple and extensive exfoliation occurs, exposing large areas to bacterial infections. Viral excretion persists for a longer period of time, resulting in more infected cells, hence more severe disease (Walsh, 1983). A study of 69 children suggested that there is a genetic predisposition for the development of a severe form of the disease (Coovadia *et al.*, 1981). A significant excess of histocompatibility antigen (HLA) AW32 was observed in the patients with severe measles as compared to controls . More recent scrutiny of earlier data has suggested that overcrowding and intensive exposure to measles virus may increase measles mortality and may be more of a risk factor than the nutritional status of the child (Aaby *et al.*, 1988). Measles is also a major cause of death in children with malignant diseases (Gray *et al.*, 1987).

D. MV interaction with PBMC

1. In vivo

MV dissemination throughout the body, prior to the onset of disease, occurs via PBMC following the virus replication in lymphoid tissues (Burnet, 1968). The presence of the virus in PBMC and lymphoid tissues of measles patients has been clearly shown (White *et al.*, 1973; Osunkoya *et al.*, 1974; Hyypiä *et al.*, 1985; Moench *et al.*, 1988). Phytohemagglutinin (PHA) stimulation of PBMC, obtained from children with measles, resulted in the formation of large multi-nucleated (giant) cells (Osunkoya *et al.*, 1974a; 1974b). MV antigens could also be detected in PHA-stimulated PBMC, whereas unstimulated cultures were negative. These results indicate that MV not only infects human PBMC but may also proceed to active replication when PBMC are activated (Osunkoya *et al.*, 1974a; 1974b). Formation of large syncytia of thymocytes, resulting in partial or complete loss of the thymic cortex, depending on the severity of the disease, has been reported (White *et al.*, 1973). These observations may explain the leukopenia occurring during measles (Coovadia *et al.*, 1978). Significant depression of the total T cell count has also been observed (Whittle *et al.*, 1978; Joffe *et al.*, 1983; Alpert *et al.*, 1984). A decrease in the inducer/helper T cells (defined by OKT4 antiserum) resulting in decreased ratios of inducer/helper to suppressor/cytotoxic T cells (OKT4:OKT8) has been observed (Joffe *et al.*, 1983; Alpert *et al.*, 1984) but other reports have failed to confirm these observations (Arneborn *et al.*, 1983; Hirst *et al.*, 1984; Griffith *et al.*, 1986).

A severe but transient immunosuppression is observed during measles. The first report of MV-induced immunosuppression dates back to 1908, when von Pirquet observed a decrease in tuberculin skin tests in patients suffering from measles (von Pirquet, 1908). Since that time, the report has been confirmed and extended to *in vitro* studies (Starr *et al.*, 1964; Zweiman, 1971; Lucas *et al.*, 1978; Ilonen *et al.*, 1988). MV inhibitory effect on the immune response has a profound effect on the body. It can lead to secondary infections which, if left untreated, can be fatal (Orren *et al.*, 1981). The immunosuppression can aggravate pre-existing conditions, such as tuberculosis, as was first noted by William Osler in 1892. He noticed the dissemination or reactivation of tuberculosis in the course of measles infection (Osler *et al.*, 1892). MV infection can also help in the remission of various autoimmune diseases such as rheumatoid arthritis and asthma (Simpanen *et al.*, 1977; Yoshioka *et al.*, 1981). The severity of measles immunosuppression may distinguish children who will recover normally from children who will die or develop complications (Coovadia *et al.*, 1978). In a study of 60 children with measles, 77% of 30 of those children with severe lymphopenia during measles subsequently died or progressed to chronic chest disease whereas 67% of the remaining children with less severe lymphopenia, recovered normally (Coovadia *et al.*, 1978).

2. IN VITRO

In vitro studies have shown that MV can infect B cells, T cells, and monocytes/macrophages but not polymorphonuclear leucocytes (PMN) (Joseph *et al.*, 1975; Huddlestone *et al.*, 1980). The infection is non-productive in unstimulated PBMC and becomes productive upon stimulation of the cells (Lucas et al., 1978). Interaction of MV with PBMC has been shown to decrease the cell response to various stimuli, such as mitogens, antigens and allogeneic lymphocytes (Zweiman, 1971; Sullivan *et al.*, 1975; Lucas *et al.*, 1978; Ilonen *et al.*, 1988), but the effects vary depending upon the virus strain used, the multiplicity of infection and the lymphocyte donor as well as the concentration of antigens or mitogens used (Dunmire, 1975; Graziano *et al.*, 1975; Ilonen *et al.*, 1988). The immunosuppressive effect of MV may occur as a result of the killing of PBMC by the virus. This is supported by the fact that measles patients suffer from leukopenia (Coovadia *et al.*, 1978; Wesley *et al.*, 1978; Whittle *et al.*, 1978; Borysiewick *et al.*, 1985). However, it has been demonstrated that MV can inhibit lymphocyte mitogenesis *in vitro* without a decrease in cell viability (Sullivan *et al.*, 1975). In a more recent study, it has been proposed that cell death may be involved in the inhibitory effect of the virus, the extent of which depends upon the viral strain used (Salonen *et al.*, 1989). The authors showed that MV inhibition of PBMC proliferation can be divided into an early inhibition and a late one. The early inhibition is partially mediated by interferon α (IFNα), while the late inhibition is caused by MV-induced death of the responding cells (Salonen *et al.*, 1989).

The role of the specific subsets of PBMC has also been investigated. Earlier reports have failed to attribute any role to the monocyte/macrophage cell populations (Sullivan *et al.*, 1975; Lucas *et al.*, 1978). Using peripheral blood cell cultures depleted of adherent cells, Sullivan and coworkers were unable to detect any change in the inhibitory effect of the virus. Using mixed lymphocyte reactions, Lucas and coworkers found that MV was still inhibitory when infected lymphocytes were cultured in the presence of measles antibodies and uninfected monocytes (Lucas *et al.*, 1978). However, more recent studies have suggested that monocytes may play a role in the immunosuppressive effect of the virus. These deductions were based on the fact that rigorous depletion of monocytes led to a partial restoration of the response by lymphoid cells. Also, T cell lines lacking monocytes were inhibited only late after infection, coinciding with a decrease in the number of living cells (Salonen *et al.*, 1989). It has also been reported that viral genes are expressed preferentially in monocytes as compared to T cells, in unstimulated cultures. About 80% of MV-antigen positive cells were MMA (monocyte marker) positive, in unstimulated cultures, while less than 10% were of T cell lineage (defined by OKT3 antiserum). Higher yields of infectious virus were obtained from the MMA positive cells than from OKT3 positive cells (Salonen *et al.*, 1988). In a study of 51 measles patients, it was found that monocytes contribute to the depressed proliferative response occurring during measles (Griffith *et al.*, 1987). Decreasing the number of adherent cells increased the proliferative response in some patients. However, in other patients, the role of adherent cells was found to be of lesser importance (Griffin *et al.*, 1987).

The role of immune mediators, such as the interleukins and the interferons (IFNs) in MV-induced immunosuppression has also been studied. Interleukin-2 (IL-2), an essential component of immune responses, is affected during some viral infections, e.g, RSV, Sendai virus, herpes simplex virus (HSV) and influenza A virus (Borysiewick *et al.*, 1985; Mims, 1986). It has been suggested that avian retroviruses can complex directly with IL-2 and inactivate it (Wainberg *et al.*, 1983; Wainberg *et al.*, 1984). This phenomenon resulted in the inhibition of mitogen-induced lymphocyte proliferation, which can be restored by the addition of IL-2, suggesting that IL-2 receptors are not affected by the virus. Infection of lymphoid cells with HSV or influenza A virus leads to a partial decrease in IL-2 production upon stimulation by PHA (Borysiewick *et al.*, 1985). A marked decrease in IL-2 production was observed when RSV and Sendai virus were used. However, the level of IL-2 was not affected in MV-infected PBMC (Borysiewick *et al.*, 1985; Salonen *et al.*, 1988).

Viruses are potent inducers of IFNs (Joklik, 1985). IFNs are best known for their antiviral effects both *in vitro* and *in vivo*. They also have a wide range of other biological activities, including inhibition of cell replication and changes in cell metabolism (Gresser, 1977). Cells of the immune system are more affected by the anticellular activity, which can result in immunosuppression, as has been shown in many laboratories (De Maeyer *et al.*, 1975; Johnson *et al.*, 1975; Sonnenfeld *et al.*, 1977). Injection of IFN or IFN-inducers have been shown to cause lymphopenia in mice (Schacter *et al.*, 1983). Upon treatment with anti-IFN α/β, the lymphopenia was eliminated. IFNs can also induce immunosuppression *in vivo* (Brenan *et al.*, 1983). Infection with viruses that induce high levels of IFN, diminishes virus specific responses to a second virus infection. Antibodies to IFN can reverse that effect (Brenan *et al.*, 1983). The role of IFN, if any, in MV-induced immunosuppression has yet to be clearly demonstrated. *In vitro* studies by Lucas and coworkers, have failed to detect significant amounts of IFN in MV-infected, mitogen stimulated cultures and mixed lymphocyte cultures (Lucas *et al.*, 1978). However, Ilonen and co-workers detected IFNα, ranging in values from 400 IU/ml to 6400 IU/ml, in cultures infected with different strains of MV

(Ilonen *et al.*, 1988). But no correlation was found with "...either the proliferation inhibition, the release of infectious virus or the strain of infecting virus". While some studies have attributed a significant role to IFNα in MV-induced suppression (Neighbour *et al.*, 1979; Salonen *et al.*, 1989), others have failed to do so (Lucas *et al.*, 1978; Sanchez-Lanier *et al.*, 1988). Other reports have detected the presence of a DNA synthesis inhibitory molecule, but have failed to identify it (Minagawa *et al.*, 1974; Whittle *et al.*,1978).

E. MV persistence

Persistence is one among many of the strategies used by viruses for survival. It has important implications for the host as it can lead to a severe disease following reactivation, or by the continuous shedding of viral antigens. Immunopathological disease may result from immune reactions to the virus present in the body (Mims, 1978; 1986). In humans, persistence of MV can lead to SSPE (ter Meulen *et al.*, 1982). Persistent MV infection has also been implicated as the etiology for certain other diseases, such as multiple sclerosis (Adams *et al.*, 1962), Paget's bone disease (Basle *et al.*, 1985) and autoimmune chronic active hepatitis (Robertson *et al.*, 1987).

Persistence of MV is a well documented phenomenon (Minagawa *et al.*, 1976; Rima *et al.*, 1976; Rima *et al.*, 1977; Jacobson *et al.*, 1982). It occurs both *in vivo* and *in vitro*, in nature or experimental conditions. The mechanism(s) by which MV can persist in the midst of competent immune cells is not known. Several explanations for the persistence have been proposed. A decrease in the amount of viral antigens expressed on the cell surface during persistency is one explanation. Such cells are not lysed by the immune cells, due to lack of sufficient amount of viral antigens on their surface, ensuring the presence of the viral genome within the body. Alternatively, the antigens may be removed from the cell surface by antibodies resulting in a cell denuded of viral antigens (Joseph *et al.*, 1975; Fujinami *et al.*, 1981; Oldstone *et al.*, 1982; Fujinami *et al.*, 1984).

IFNα is an important host-derived factor involved in persistence (Jacobson *et al.*, 1982, Rima *et al.*, 1976). It has been suggested that IFNα is responsible for persistence of the virus in unstimulated PBMC (Jacobson *et al.*, 1982). The unstimulated blood cells not only produce a large amount of IFN, but treatment of the infected cells with anti-IFN serum results in a productive infection. Furthermore, no defective interfering particles were detected in those experiments (Jacobson *et al.*, 1982).

Defective interfering particles and temperature sensitive (ts) mutants have been suggested as the two main strategies for achieving persistency (Rima *et al.*, 1976; ter Meulen *et al.*, 1982). The involvement of defective interfering particles in persistency has been described for several viruses (Welsh *et al.*, 1972; Kimura *et al.*, 1975; Holland *et al.*,1980), including MV (Rima *et al.*, 1977). Establishment of persistent infection of Vero cells by MV Edmonston was readily obtained with inocula passaged serially without dilution. However, when the inoculum was plaque purified, infection did not result in persistency (Rima *et al.*, 1977). It has earlier been shown that undiluted passages of MV results in an accumulation of defective interfering particles (Hall *et al.*, 1974; Kiley *et al.*, 1974) which are eliminated by plaque purification (Kiley *et al.*, 1974). Furthermore, it has also been shown that the accumulation of defective interfering particles during passage of measles virus led to a decrease of virus-specific proteins synthesized in infected cells (Rima *et al.*, 1979).

It has been suggested that ts mutants play a role in the establishment (Gould *et al.*, 1975; Minagawa *et al.*, 1976) or maintenance of persistent infections (Preble *et al.*, 1973). *In vitro* studies have shown that MV ts mutants are involved in persistency (Norrby, 1967; Knight *et al.*, 1972; Haspel *et al.*, 1973). The rate of MV production in persistently infected cells of diploid human embryonic lung markedly increased at 33°C relative to 37°C (Norrby, 1967). A 100-fold increase in virus maturation was observed when hamster embryo fibroblasts, persistently infected with MV, were grown at 33°C compared to 37°C (Knight *et al.*, 1972). At 39°C, virus maturation was completely inhibited. In a subsequent study, it was shown that maturation of MV in these cells was temperature sensitive (Haspel *et al.*, 1973). In a study by Ju *et al.* (1978), 73% of all virus isolates obtained from four persistently infected lymphoid cell lines early in infection were found to be ts mutants. This led the authors to conclude that "...the appearance of ts mutants was directly associated with the establishment of the persistent infection" (Ju *et al.*, 1978; 1980).

F. Rationale of the work

The fact that MV persistency can lead to SSPE; and that MV ts mutants can induce persistency, has prompted several studies on the interaction of MV ts mutants with permissive cells, at the restricted temperature. However, in spite of the fact that PBMC are the primary target cells for MV, few studies have focused on the interaction of MV ts mutants with PBMC; of those that did, only continuous cell lines were used. The main reason is that, for any infection to be considered persistent, the virus must remain within the host for a long period of time, thus requiring the use of long term cultures.

Ts mutant infection of permissive cells at the restrictive temperature may lead to abortive infections. One of the consequences of an abortive infection is persistence. Thus, an abortive infection induced by a ts mutant in a permissive cell may be considered to be the initial step towards persistency. However, the interaction of MV ts mutants with PBMC under abortive conditions has never been investigated. In this work, the interactions of a ts mutant of MV (MV ts38), isolated in Vero cells, with PBMC was studied and the following questions were addressed:

1. Were the ts properties of MV ts38 retained in PBMC?

2. Did MV ts38 induce an abortive infection in PBMC at the restricted temperature? If so, at what level was MV ts38 replication blocked?

3. Was PBMC proliferation in response to different stimuli affected during an abortive infection by MV?

4. What was the relative importance of monocytes and T cells in MV ts38-inhibitory effect on PBMC proliferation?

5. Was there preferential replication of MV in T cells or monocytes?

6. Was cell death a prerequisite for MV to be inhibitory?

7. Was the level of interleukin-2 affected by MV infection and was IFNα involved in the inhibitory effect exerted by MV?

8. Was a DNA synthesis inhibitory molecule present? If so, what were its properties?

Materials and Methods

A. Cell culture media and reagents

Growth medium for monolayer cultures of Vero, HeLa, HEp-2 and MDBK cells was Eagle's minimal essential medium (MEM) with Earle's salts (Gibco Laboratories Inc. Burlington, Ontario) supplemented with 1% L-glutamine 0.2% sodium bicarbonate, 5% fetal bovine serum (FBS) (Flow laboratories Inc. Virginia, USA), 100 IU of penicillin and 0.1 mg of streptomycin sulphate per mL.

Growth medium for peripheral blood mononuclear cells and subsets was RPMI 1640 (Gibco Laboratories Inc. Burlington, Ontario) supplemented with 1% L-glutamine, 10% FBS, penicillin and streptomycin as described above. Phosphate buffered saline (PBS) was prepared according to standard techniques (Schmidt, 1979).

B. Cell cultures

Vero cells (African green monkey kidney cells, ATCC number CCL 81), HeLa cells (derived from human cervix epithelioid carcinoma, ATCC number CCL 2), HEp-2 cells (derived from human larynx epidermoid carcinoma, ATCC number CCl 23) and MDBK cells (bovine kidney cells, ATCC number CCL 22) were obtained from the American Type Culture Collection (ATCC) (Rockville, Maryland). Cultures were fed twice weekly and maintained at 37°C in an atmosphere of 5% CO_2. Each cell line was passaged every 3 to 5 days. Monolayers were washed with PBS, trypsinized at room temperature and cultured in the growth medium, as described in section A, in either roller bottles, flasks or small tissue culture plates depending on the intended use.

Culture supernatant solutions obtained from hybridoma cell lines (ATCC numbers CRL 8001 and HB 78 respectively) were used as a source of anti-OKT3 and MMA monoclonal antibodies. The hybridoma cells were purchased from the ATCC (Rockville, Maryland).

C. Isolation of PBMC

Human peripheral blood mononuclear cells (PBMC) were obtained from the Red Cross Blood Transfusion Center , Edmonton, Alberta in the form of buffy coats. PBMC were isolated on Ficoll Hypaque gradients (Böyum et al., 1968) according to the supplied protocol (Pharmacia Fine Chemicals, Piscataway, NJ). The resultant cells were washed three times in PBS, suspended in culture medium and counted in a hemocytometer. Cell viability determined by the Trypan Blue exclusion procedure, was always greater than 98%.

D. Enrichment of monocytes

Monocyte-enriched populations were obtained in one of two ways: PBMC, at a concentration of 5 x 10^6/mL, in FBS-supplemented RPMI 1640 were incubated in a tissue culture plate for 45 minutes at 37°C. Following this, the supernatant solution (SN) was removed and the plate was washed three times with RPMI 1640. The adherent cells were removed by gently scraping with a rubber policeman or by the addition of 2 mM EDTA. The adherent cells were then washed twice with PBS and incubated for 30 minutes on ice with anti-OKT3 antibodies. The cells were washed twice and incubated for an hour at room temperature with 0.5 mL of rabbit complement (Cedarlane laboratories, Hornby, Ontario). After three more washes in PBS, the cells were suspended in RPMI 1640 supplemented with 10% FBS and counted.

The second method made use of a Percoll discontinuous density gradient (Fluks, 1981) which was prepared as follows: A stock solution of Percoll was prepared by mixing 90 mL of Percoll (Pharmacia Fine Chemicals, Piscataway, NJ.) together with 10 mL of 10 x concentrated Hanks balanced salt solution and the pH was adjusted to 7.4, using 1 N HCl. To obtain the desired buoyant densities (ρ), 10 mL of the stock solution was diluted with FBS-supplemented RPMI as follows: for ρ 1.060, 10.5 mL of RPMI was added; for ρ 1.062, 9.8 mL was added; for ρ 1.066, 8.6 mL was added; 7.6 mL and 5.4 mL were used for ρ 1.070 and 1.080 respectively. A cell pellet containing 5 x 10^7 PBMC was suspended in 5 mL of the solution of ρ 1.080 and the mixture was placed in a 15 mL centrifuge tube. Two mL of each of the solutions of different densities was layered on top of the cell suspension and the gradients were centrifuged for 30 minutes at 400 x g at room temperature. The top fraction containing the monocytes was collected and the cells were washed twice in PBS. MMA monoclonal antibodies (specific for monocytes) were used to determine the purity of the cells (Hanjan *et al.*, 1982).

E. Enrichment of T cells

Enriched T cell populations were prepared in two different ways: PBMC were passed through the plastic attachment procedure twice as described above (section D) and the resulting non-adherent cells were washed once in PBS and incubated on ice for 30 minutes with MMA antibodies. The cells were then washed twice with PBS and incubated with rabbit complement as described earlier (Section D). The cells were washed twice in PBS and viability was assessed by Trypan Blue exclusion.

In the second method, the non-adherent cells were purified by the use of nylon wool column (Julius et al., 1973). Nylon wool in LP-1 Leuko Pak leukocyte filters (Fenwal Laboratories, Morton Graves, Illinois) was soaked for two days in deionized double distilled water. Nylon wool was then packed into a 10 mL syringe up to the 7 mL mark. The columns were autoclaved and stored until used. Prior to use, the columns were soaked in RPMI 1640 medium supplemented with 10% FBS at 37°C for 30 minutes. One to 2 x 10^8 cells in 2 mL were applied onto each column and incubated for 1 h at 37°C. The columns were then washed with 20 mL of the same medium and the effluent was collected. Anti-OKT3 antibodies were used to determine the purity of the cells.

F. Proliferation assays

Ninety six-well round bottom plates were used for proliferation assays. To each well, 5 x 10^4 PBMC or subsets of cells were added together with different chemicals, as described in the figure legends, in a total volume of 250 µL. PHA and lipopolysaccharrides (LPS) (Sigma, Chemical Co. St Louis, Missouri) when used, were added to a final concentration of 10 µg/mL and 25 µg/mL, respectively. The optimal stimulatory concentration of both mitogens was determined prior to use. Plates were incubated at the specified temperatures in a 5% CO_2 atmosphere. After 54 to 56 h, 14.8 KBq of [^{3}H]-thymidine (74 GBq/mmol, New England Nuclear Research Products, Boston) was added to each well and the contents were harvested 16 to 18 h later on glass wool filters using the Skatron cell harvester (Skatron, Inc. VA). For each test, six parallel wells were used. Thirty seven KBq of [^{3}H]-thymidine was added when radioactive labeling was carried out for only 6 h.

For some experiments, PBMC or subsets of cells at a concentration of 1 x 10^6/mL were cultured in 15 mL or 50 mL tissue culture tubes in a 5% CO_2 atmosphere at the desired temperature.

G. Mixed lymphocyte reactions

PBMC were prepared and infected as described in section C and H. In one-way mixed lymphocyte reactions, 5 x 10^4 stimulator cells irradiated with 2000 rads, and 5 x 10^4 MV-infected allogeneic cells were cultured in 200 µL. [^{3}H]-thymidine was added for the last 18 to 20 h of a total incubation period of 5 or 6 days at 37°C.

In two-way mixed lymphocyte reactions, 5 x 10^4 MV-infected PBMC were mixed with an equal amount of allogeneic

MV-infected PBMC. [^{3}H]-thymidine was added for the last 18 h of a total incubation period of 5 or 6 days at 37°C.

H. Preparation of the virus stocks and infection of cells.

The different measles virus strains used were obtained from the following sources: Edmonston (ATCC, Rockville, Maryland); VSC (Wild-type isolate); Bray, Berg, Mantooth and Woodfolk (from Dr. W. Hall, Rockerfeller University, New York.); Halle (from Dr. T. F. Wild, INSERM, Lyon, France); Lec KI (from Dr. E. Norrby, Karolinska Institute, Stockholm, Sweden) and MV ts38 (a laboratory induced mutant, originally isolated in our laboratory,) (Chui *et al.*, 1986). All the cultures were propagated in roller bottle cultures of Vero cells at 32°C. The virus inoculum, in a total volume of 5 mL, was added to confluent monolayers at a multiplicity of infection (m.o.i.) of 0.1 to 0.01. After one hour adsorption at 37°C, MEM supplemented with 5% FBS was added and the cells were incubated at 32°C. At the time of maximal CPE, the supernatant solutions were clarified by centrifugation at 400 x g for 10 minutes and frozen in small aliquots at -70°C. Virus stocks were assayed by plaque formation in Vero cells. Vesicular stomatitis virus (VSV), Indiana strain, (Salonen et al., 1982) was grown in Vero cells at 37°C, as described above.

MV at an m.o.i. of 3 to 5 was used to infect PBMC and subsets. Cells were incubated with the virus inoculum in a total volume of 0.2 mL for one hour at 37°C, the cells were sedimented by centrifugation and the SN was removed. The cells were then washed twice with PBS and fresh medium was added.

In some experiments, the cells were added to microtiter plates first and then infected with virus. Following adsorption, the plates were centrifuged and the cells were washed twice with PBS to remove unbound virus and fresh medium was added.

I. Plaque assays

One hundred µL of tenfold dilutions of samples to be tested were added to one day old monolayers of Vero cells in 12 well plates (Flow laboratories Inc. Mississauga, Ontario). After one hour's adsorption at 37°C with frequent shaking, 2 to 3 mL of an overlay was layered onto the cell monolayer. The overlay solution consisted of a 0.5% solution of carboxymethyl cellulose (Sigma, Chemical Co. St Louis, Missouri) in MEM supplemented with 100 IU penicillin/mL, 0.1 mg of streptomycin/mL, 2 mM glutamine, 0.1 % sodium bicarbonate and 1.5 mM HEPES buffer. The plates were incubated for 6 to 7 days in a humidified chamber with 5% CO_2 at 37°C. For MV ts38, similar plates were incubated at 32°C and 39°C for 8 to 9 days.

A second method using an agar overlay was also used. Cell cultures were prepared and infected with virus as described above. The overlay solution consisted of 0.6% Noble agar in MEM supplemented with 2mM glutamine, 100 IU penicillin/mL, 0.1 mg streptomycin/mL and 5% FBS together with an equal volume of 1.2% Noble agar (Difco Laboratories, Michigan, USA) in water. Two to three mL of the overlay solution was added to each well and the plates were incubated at the required temperature for a period of 7 to 9 days. Cells, in both cases, were fixed with 10% acetic acid overnight followed by staining with a solution containing 0.2% crystal violet in 5% formaldehyde and 10% ethanol. Plaques were counted and infectivity was expressed as pfu/mL.

J. RNA isolation

1. Guanidium isothiocyanate method

Vero cells were infected at an m.o.i. of 1 and PBMC or subsets of cells were infected at an m.o.i. of 3 to 5. The cells were cultured as described earlier (sections B and H). Twenty four to 36 h post infection, the Vero cells were washed with cold PBS and scraped off the surface of the dishes. Cells were collected into siliconised 50 mL tubes and homogenized in 4 M guanidine isothiocyanate (Fluka, Switzerland) in the presence of 0.1% (w/v) β-mercaptoethanol (Maniatis et al., 1982). The homogenate was passed through an 18.5 gauge needle. The resulting mixture was centrifuged at 5000 x g for 20 minutes at 4°C in a Sorvall SS34 rotor. The SN was layered onto a CsCl step gradient consisting of 1 mL of 2.4 M CsCl and 3 mL of 5.7 M CsCl (Pharmacia Fine chemicals, Piscataway, NJ) and centrifuged at 100,000 x g in an SW40 rotor for 24 h at 20°C. Following centrifugation, the SN was removed and the remaining pellet was dissolved in 1 mL of a solution consisting of 5% SDS, 10 mM TrisHCl (pH 7.5) and 1 mM EDTA (pH 7.5). Sodium chloride was added to a final concentration of 0.1 to 0.15 M from a stock solution of 5 M and the resulting mixture was warmed in a 65°C water bath for 3 minutes and rapidly cooled on ice. Extraction with one volume of phenol, chloroform, isoamyl alcohol in the ratio of 25:24:1 was carried out, followed by precipitation overnight in 95% ethanol containing 0.3 M sodium acetate, pH 5.2. Samples were centrifuged in an Eppendorf microfuge at 4°C for 30 minutes and the resulting pellets washed in 70% ethanol containing 125 mM sodium acetate. The mixture was incubated at -70°C for 10 minutes, warmed to room temperature and centrifuged in an Eppendorf microfuge for 30 minutes. Pellets were air-dried and dissolved in Tris-EDTA (TE) (10 mM TrisHCl, pH 7.5 and 1 mM EDTA) buffer. Samples were stored at -70°C in 70% ethanol with 125 mM sodium acetate until used.

2. Rapid method

A rapid method was also used for RNA isolation (Glickman *et al.*, 1988). Cells were harvested and washed in PBS. One mL of lysis buffer, consisting of 150 mM NaCl, 10 mM TrisHCl, pH 7.9 and 1% NP40, was added to a pellet of 2×10^7 cells and the mixture was incubated on ice for 5 minutes. The homogenate was passed through an 18.5 gauge needle and centrifuged in an Eppendorf microfuge for 5 minutes at 4°C. The SN was harvested and mixed with an equal amount of chilled (4°C) urea buffer (10 mM TrisHCl, pH 7.5, 10 mM EDTA, 350 mM NaCl, 7 M urea and 1% SDS). The solutions were thoroughly mixed and extracted with phenol: chloroform:

isoamyl alcohol three times and precipitated with ethanol, as described above (section J-1). The pellets, obtained after centrifugation, were dissolved in TES (10 mM TrisHCl, pH 7.5, 5 mM EDTA and 0.5% SDS), 0.2 mg/mL of proteinase K (Sigma, St Louis, Missouri) was added and the solution was incubated for 30 minutes at 37°C. The samples were extracted and precipitated (section J1). The pellets were dissolved in TE buffer. Samples were stored at -70°C, until used.

K. RNA electrophoresis and Northern blots

RNA pellets, dissolved in denaturing buffer consisting of 50% deionized formamide, 1 x morpholinopropanesulfonic acid (MOPS)/EDTA and 6% formaldehyde, were kept at 65°C for 15 minutes and then quickly chilled on ice. One tenth volume of a dye mixture consisting of 0.1% Bromophenol Blue, 0.1% xylene cyanole, 35% Ficoll and 0.5% SDS, was added to each sample. The samples were applied to a formaldehyde-agarose gel (6% formaldehyde and 1 to 1.2% agarose) which had been pre-equilibrated at 60 V for 30 minutes using 1 x MOPS/EDTA as running buffer. The samples were electrophoresed for 4 to 5 h at 100 V. The lanes containing standards were cut and stained with ethidium bromide (1 mg/mL).

For transfer to nitrocellulose or nylon membranes, gels were washed three times (10 minutes per wash) in distilled water, treated with 50 mM NaOH for 30 minutes, 0.1 M TrisHCl pH 7.5 for 30 to 40 minutes, and with 10 x SSC (1.5 M sodium chloride and 0.15 M sodium citrate, pH 7.0) for 30 to 40 minutes. Nitrocellulose paper or nylon membranes were cut to gel size and soaked in distilled water for 2 to 3 minutes followed by 2 x SSC for at least 15 minutes. The gels were blotted for 20 to 24 h at room temperature and rinsed for 5 to 8 minutes in 6 x SSC. The membranes were baked at 60°C overnight and stored at -20°C until used.

For dot blots, RNA suspended in TE buffer was mixed with 50% formamide and 6% formaldehyde in a total volume of 200 µL. The mixture was incubated at 50°C for one hour and then chilled on ice. The mixture was then spotted by using a Hybridot apparatus (Bio-Rad Laboratories, Richmond, Calif.) onto nitrocellulose or nylon membrane that was prepared as described above. The blots were air dried and baked for 2 h at 80°C.

For slot blots, both samples and membranes were treated as described for dot blots except that 100 µL of sample was spotted per well of a slot blot apparatus (Bio-Rad Laboratories, Richmond, Calif.).

L. Single stranded probe preparations

cDNA clones of measles virus N, P, M, F, and H were provided by Dr. W. Bellini (CDC, Atlanta, Georgia). The clones were obtained as transformed *E. coli* HB101 cells containing PBR 322 vectors with MV gene fragments inserted in the *Pst* I site. These cells were grown on yeast-extract tryptone (YT) agar plates in the presence of 15 µg/mL of tetracycline. Single colonies were picked following overnight incubation at 37°C. Ten mL cultures were grown overnight at 37°C in Luria broth (LB) supplemented with 15 µg/mL of tetracycline. Five mL of the overnight cultures were transferred to 500 mL of M-9 medium containing 15 µg/mL of tetracycline. The M-9 cultures were grown at 37°C for 6 to 8 h followed by overnight incubation in the presence of 50 µg/mL of chloramphenicol. The cells were harvested by centrifugation at 5,000 x g in a sorvall GSA rotor at room temperature for 20 minutes and then washed in cold TNE (10 mM TrisHCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, pH 8.0). The cell pellets were suspended in 3.5 mL of solution I (50 mM glucose, 10 mM EDTA, pH 8.0, 0.25 M TrisHCl, pH 8.0 and 15 mg/mL lysozyme) and incubated on ice for 30 minutes. Seven mL of solution II (0.2 N NaOH + 1% SDS) was then added, gently mixed, and incubated on ice for 10 minutes. After addition of 5.5 mL of solution III (3 M sodium acetate, pH 5.2), the lysate was incubated on ice for an hour and centrifuged at 13,000 x g for 15 minutes at 4°C in a Sorvall SS34 rotor. The SN were precipitated as described earlier (section J1). The SN were centrifuged at 10,000 x g for 10 minutes at 4°C and the pellets were suspended in 15 mL of TE (50 mM TrisHCl, pH 8.0 and 10 mM EDTA). Cesium chloride (1 mg/mL) and ethidium bromide (0.6 mg/mL) were added and the samples transferred to Quick seal tubes. Paraffin oil was used to fill the tubes before sealing. Centrifugation at 150,000 x g for 20 to 24 h at 20°C in a R75Ti rotor was carried out. The band containing the plasmid DNA was removed and ethidium bromide was extracted from the DNA with n-butanol (5 to 6 extractions). The samples were dialysed against TE (10 mM TrisHCl, pH 8.0

and 10 mM EDTA) and extracted as described previously (section J1). After centrifugation at 9,000 x g in a Sorvall SS34 rotor for 30 minutes at 4°C, the pellets were washed with 70% ethanol containing 125 mM sodium acetate, air-dried and suspended in TE buffer.

For isolation of gene fragments, 2 µg of isolated DNA was incubated at 37°C overnight in the presence of 14 U of *Pst* I (Boehringer Mannheim, Canada). Electrophoresis was performed on 0.8% agarose gels (BioRad LMP agarose) in TBE (0.89 M Tris borate, 0.089 M Boric acid and 0.002 M EDTA) buffer at 50 mA using a BioRad mini gel apparatus (Bio-Rad Laboratories, Richmond, Calif.). Both gel and buffers contained 0.3 to 0.5 µg/mL of ethidium bromide . The desired bands were cut under UV light and heated for 30 minutes at 70°C, together with 200 µL of TE buffer. DNA was purified by phenol followed by phenol, chloroform and isoamyl alcohol (25:24:1) and chloroform and isoamyl alcohol (24:1) at room temperature, precipitated as described above (section J1) and dissolved in TE buffer.

For the generation of single stranded probes, isolated fragments were cloned into M13 vectors as follows: 50 ng of isolated fragments were incubated with 10 to 15 ng of *Pst* I-cut M13mp18 or mp19 that had been pretreated with bacterial alkaline phosphatase (BAP) for 2 to 3 h at 16°C. Table 1 below illustrates the way the experiment was carried out. *E. coli* JM107 cells were transformed with the processed DNA as

Table 1. Conditions used for ligation reactions in the preparation of single stranded probes using MV genes.

# treatment	5x Lig. buffer (μL)	Vector DNA (μL)	Sample DNA (μL)	dATP 1mM (μL)	Ligase 1U/μL (μL)	dH_2O (μL)	Total Vol (μL)
1a Uncut vector	2	2(18)	-	-	-	6	10
1b	2	2(19)	-	-	-	6	10
2a Cut vector	2	2(18)	-	-	-	6	10
2b	2	2(19)	-	-	-	6	10
3a Cut & ligated	2	2(18)	-	1	1	4	10
3b vector	2	2(19)	-	1	1	4	10
4a Cut, BAP treated	2	2(18)	-	1	1	4	10
4b & ligated	2	2(19)	-	1	1	4	10
5a Cut & sample	2	2(18)	3	1	1	1	10
5b ligated	2	2(19)	3	1	1	1	10
6a Cut, BAP treated	2	2(18)	3	1	1	1	10
6b sample ligated	2	2(19)	3	1	1	1	10

Controls: 1. Uncut vector (m13 RF DNA); 2. Cut vector (m13 RF/enzyme cut); 3. Cut and ligated (enzyme/T4 DNA ligase); 4. Cut, BAP treated and ligated

Experimental: 5. Cut vector + sample DNA + Ligase (mp18/19); 6. Cut, BAP treated + sample DNA + ligase (mp18/19) follows: 1 mL of an overnight culture of HB101 cells grown in LB medium was added to 40 mL of fresh LB medium and grown at 37°C until the optical density at a wavelength of 550 nm was 0.5. The cells were quickly chilled by adding frozen LB medium, incubated on ice and centrifuged at 2500 x g for 15 minutes at 4°C. The resulting cell pellets were suspended in ice-cold 50 mM $CaCl_2$, centrifuged and incubated for 20 minutes on ice. The pellet was suspended in 1/50 volume of 50 mM $CaCl_2$. Three hundred µL of these cells were mixed with 0.5 µg of the desired DNA, incubated on ice for 40 minutes and heat-treated at 42°C for 2 minutes. The transformation mixture was gently mixed with 0.2 mL of *E. coli* JM107 cells grown in YT medium. Three to 4 mL of top agar (YT + 1.2% agar) was added together with 10 µL of 100 mM isopropyl-β-thiogalactoside and 50 µL of 2% 5-bromo-4-chloro-3-indoxyl-β-D galactoside. The resulting mixture was poured onto YT agar plates, spread evenly and incubated at 37°C overnight. Clear plaques were picked and mixed with 2 mL of exponentially growing JM107 cells. The mixture was shaken for 3 h at 37°C. One hundred µL of the mixture was added to 1 mL of YT medium. The mixture was shaken for 6 h at 37°C, centrifuged at 3000 x g and the SN was harvested. Template DNA was prepared by adding 1.2 mL of SN to 300 µL of polyethylene glycol/2.5 M NaCl and the mixture was incubated at room temperature for 15 minutes. The mixture was then centrifuged for 10 minutes in a microfuge and the SN was carefully removed. The pellet was suspended in 100 µL of TNE. Extraction and precipitation were carried out as described previously (section J1).

M. Determination of orientation of cloned DNA

Clones from each MV gene fragment were placed in two groups depending on the orientation of the inserted MV gene fragments. To determine the orientation of the fragments, 20 µL of SN from M13-infected JM107 cultures were mixed with 5 µL of 25% Ficoll 40 containing 5% SDS and 0.025% Bromophenol Blue in a 1.5 mL microfuge tube. To the mixture, 150 ng (in 1 µL) of template DNA (obtained from an arbitrarily chosen clone with insert from the same MV gene fragment) was added. Hybridization was allowed to proceed at 65°C for one hour. The mixture was then allowed to equilibrate to room temperature. Clones with complementary DNA sequences were identified by the presence of slowly-migrating bands after electrophoresis in 0.7% agarose gels.

N. Hybridization

The single-stranded probes were labeled as follows: 170 ng of hybridization primer (Regional DNA synthesis laboratory, Calgary, Alberta) was added to 1 µg of the single stranded insert DNA, together with 1 µL of 10 x Klenow buffer and sterile distilled H_2O to a total volume of 10 µL. The contents were mixed and incubated at 65°C for 15 minutes. After the incubation, the mixture was allowed to equilibrate to room temperature and was stored at -20°C or used immediately. Ten µL of the resulting template/primer was added to 1 µL of 10 x Klenow buffer, 1 µL of 0.1 M dithiothreitol, 1 µL of deoxynucleotide mixture, 5 µL of

[^{32}P]-dCTP (101 TBq/mmol, ICN Biomedicals, Inc. Irvine, California) and 1 µL of DNA polymerase I (Klenow fragment) (Pharmacia Fine Chemicals, Piscataway, NJ.) and 1 µL of distilled water. The mixture was incubated at room temperature for 2 h. The reaction was stopped by adding 20 µL of 10 mM EDTA (pH 7.5). Ten µL of yeast tRNA (5 mg/ml) and 20 µL of Poly A$^+$ RNA (10 mg/mL) were then added. An equal volume of phenol was used to purify the DNA and the supernatant solution was collected. This was mixed with 200 µL of sodium iodide and 10 µL of glass milk (Gene Clean, BIO 101, La Jolla, Calif.) and incubated at room temperature for 10 minutes. The mixture was centrifuged and the pellet was washed four times with 600 µL of ice-cold NEW (NaCl/ethanol/water) solution (Gene Clean, BIO 101, La Jolla, Calif.). DNA was eluted twice with 150 µL of TE at 45 to 50°C for 5 minutes. The isolated DNA was mixed with 10 µL of tRNA and 500 µL of denatured salmon sperm DNA. An aliquot was used to measure the specific activity of the probe.

The blots were prehybridized for 20 to 24 h at 42°C in a solution of 5 x SSPE (5 mM disodium EDTA, 0.05 mM sodium phosphate, 0.9 M NaCl, pH 7.0) containing 50% formamide, 5% Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin), 0.1% SDS, 0.1 mg/mL Poly A$^+$ RNA, 10% dextran sulphate and 0.25 mg/mL of denatured salmon sperm DNA. For hybridization, the solution was replaced with the radioactively labeled probe at a concentration of 10^6 to 10^7

cpm/mL of a hybridization solution as described above, except that 0.05% SDS and 1% Denhardt solution were used. The blots were incubated for 20 to 24 h at 42°C and washed as follows: four washes of 15 minutes each at room temperature in 2 x SSC containing 0.1% SDS (250 mL per wash), followed by two washes of 30 minutes each at 68°C in 1 x SSC containing 0.1 % SDS (250 mL per wash) and two more washes of 30 minutes each at 68°C in 0.2% SSC containing 0.1 % SDS (250 mL per wash). The blots were air dried and exposed to Kodak X-omat AR X-ray film (Eastman Kodak Co, Rochester, NY) at -70°C in the presence of intensifying screens (Dupont Cronex Lightning Plus) (E.I. Dupont de Nemours & Co Wilmington, DE)

O. Immunofluorescence

Monoclonal antibodies to the surface markers OKT3 and MMA were used to identify T cells and monocytes, respectively. Rabbit hyperimmune serum to MV nucleocapsid protein (a gift of Dr B. Ziola, University of Saskatchewan, Saskatoon, Saskatchewan) was used to identify MV-infected cells. Labeling with these antisera was carried out at different times after infection. For surface labeling, cells were washed twice in PBS and incubated for 30 minutes in an ice bath with a 1:40 dilution of the ascites fluid obtained from hybridoma cell lines (anti-OKT3 or MMA). The cells were then washed twice with PBS. FITC-conjugated goat anti-mouse IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) was added and the incubation and washing procedures described above were repeated. For double labeling, the surface-labeled cells were centrifuged onto microscope slides with a cytocentrifuge (Cytospin 2, Shandon Instruments, Sewickley, PA, USA) and fixed in acetone at -20°C for 10 minutes. The cells were incubated with rabbit polyclonal antiserum against the nucleocapsid protein in a humidified chamber at 37°C for 30 minutes, washed as described above and incubated with rhodamine-conjugated goat anti-rabbit IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) at 37°C for 30 minutes. After further washings, the cells were examined by fluorescence microscopy. For each experiment, cells were counted three times, 100 to 200 cells being counted each time.

For immunofluorescence of MV-infected Vero cells, cells were grown on multiwell slides (Flow laboratories, Inc. Missisauga, Ontario) and infected with either MV Lec or MV ts38. Following different times of incubation at either 32°C, 37°C or 39°C, the slides were washed with cold PBS, and fixed in acetone at -20°C, for 10 to 15 minutes. The slides were either stored at -20°C or used immediately.

Monoclonal antibodies against different MV proteins, namely N, P, M, F and H (Salonen et al., 1988) were used to stain the infected cells as well as the uninfected controls. One hundred µL of monoclonal antibodies was added to each well and incubated at 37°C, in a humidified chamber for 30 minutes. The slides were washed three times in PBS. One hundred µL of FITC-conjugated goat anti-mouse antibody was then added to each well and the slides were incubated under the same conditions as described above. The slides were washed three times in PBS and a 50% solution of glycine in PBS was added. Each well was covered with a coverslip and the slides were examined with a Leitz Dialux 22 incident fluorescence microscope.

MV-infected PBMC were infected and cultured as described before (sections F and H). At the time of harvest, the cells were washed twice with PBS and, for each sample 200,000 cells in 200 µL were applied to microscope slides using the cytocentrifuge as described earlier in this section and fixed in acetone at -20°C for 10 to 15 minutes. The slides were stored at -20°C or used immediately. Before staining, the slides were allowed to reach room temperature. The slides were stained as described for MV-infected Vero cells except that 200 µL of antibodies were used.

P. Interleukin-2 (IL-2) assay

Cell-free SN were collected at 24 h and UV-irradiated to inactivate virus. An IL-2-dependent cell line, kindly provided by Dr D. Green, Department of Immunology, University of Alberta, was used at 2 x 10^4 cells/well in 96-well plates and incubated with different dilutions of the SN. The cells were incubated at 37°C for 48 h with [^{3}H]-thymidine present for the last 18 h. The cells were harvested as described earlier (section F). The incorporated radioactivity in six parallel wells at each dilution was measured by liquid scintillation spectrometry.

Q. Interferon (IFN) assay

IFN levels in culture SN were determined by a standard CPE reduction assay in microtiter plates. MDBK cells and VSV were employed as the indicator cells and test virus, respectively. A reference standard human IFNα (Interferon Sciences Ltd, New Jersey, USA) was included in the assays and the results standardized against the reference. Sheep anti-IFNα with a neutralizing titre of 450,000 neutralizing units (NU)/mL against human IFNα (3000 NU/mL against human IFNβ) was kindly provided by Professor K. Cantell, National Public Health Institute, Helsinki, Finland. Anti-IFNα was diluted in RPMI supplemented with 10% FBS to the different concentrations as indicated in the results (sections C8 and D3). Anti-IFNα was incubated for one hour at 37°C with SN to be tested. The SN were then immediately added to the cell assay as described above. Control SN were also incubated under the same conditions, with medium only (MEM supplemented with 5% FBS) or with normal sheep serum, at a dilution similar to that used for the anti-IFNα.

R. Production of supernatant solutions from MV-infected PBMC

Cultures of PBMC or subsets of cells were set up as previously described. After incubation for 24, 48 or 72 h, at 32°C or 37°C in 5% CO_2 the cells were sedimented by centrifugation, and the SN harvested. SN were UV-irradiated, and sedimented by ultracentrifugation for 3 h at 100,000 x g and assayed for infectivity. Aliquots of the SN were stored at -70°C for a minimum of three days before use.

S. Detection of inhibitory molecules by the use of continuous cell lines

Cells grown as confluent cultures in 150 cm^2 plastic tissue culture flasks were lifted from the plastic by a trypsin solution (2.5 mg/mL), washed in PBS, suspended in MEM containing 5% FBS and the number of viable cells was determined by Trypan Blue exclusion. Two hundred µL of cells (25×10^5 cells/mL) were added to each well of a 96-well flat bottom plate and incubated for 24 h at 37°C in 5% CO_2. SN from PBMC were added and [^{3}H]-thymidine at a concentration of 37 KBq/well was added 4 to 6 h later. Cultures were incubated for another 18 to 20 h at 37°C and harvested. The cells were washed with PBS, rinsed with trypsin, and incubated at 37°C with 200 µL of trypsin (2.5 mg/mL)/well. The cells were harvested onto glass fiber filters using the Skatron cell harvester and the amount of incorporated radioactivity was measured by liquid scintillation spectrometry.

T. Antibody treatment of supernatant solutions

SN were centrifuged for 3 h at 100,000 x g and incubated at 37°C with polyclonal antiserum against MV or normal rabbit serum, for 1 hr. The SN were centrifuged for 3 h at 100,000 x g. The control SN were similarly treated. Treated SN were tested using the cell assay described in section S.

U. Treatment of supernatant solutions at pH 2.0

SN were dialysed for 48 h against RPMI 1640 medium, brought to pH 2.0 with HCl. SN were dialysed for another 48 h against RPMI 1640 (pH 7.5). Control SN were dialysed for 96 h against RPMI 1640 (pH 7.5).

V. Immunoprecipitation

Cultures of PBMC and cell subsets were established as previously described (sections D, E anf F). These were incubated overnight for 16 to 18 h and the medium was replaced with RPMI containing one tenth of the normal concentration of methionine plus 3.7 MBq of [^{35}S]-methionine/mL. (22.75 TBq/mmol, New England Nuclear research products, Boston). The mixtures were incubated for another 16 h. The cells were washed once with PBS and mixed with RIPA buffer (0.15 M NaCl, 1% sodium deoxycholate, 1% Triton X 100, 0.1% SDS, 1 mM phenylmethyl sulphonyl fluoride and 1%

trasylol in 0.01 M Tris, pH 7.4). The mixture was incubated on ice for 2 to 3 h, followed by centrifugation for 10 minutes in an Eppendorf microfuge. If not used immediately, the SN were collected and stored at -20°C. Polyclonal antibodies against MV, at a final dilution of 1:100, were added to the SN and incubated on ice for 3 h. The immune complexes were precipitated by the addition of a slurry of protein A bound to Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ.) suspended in PBS and incubated at 4°C overnight with constant shaking. The Sepharose beads were sedimented in an Eppendorf microfuge and washed five times with RIPA buffer and once with PBS. The beads were dried and immune complexes dissociated by the addition of 100 µL of Laemmli sample buffer (2% SDS, 4% β-mercaptoethanol, 10% glycerol, 0.0625 M Tris H_3PO_4, pH 6.8). Solubilization was completed by incubating the mixtures at 100°C for 5 minutes. Fifty µL of each sample was analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

W. Polyacrylamide gel electrophoresis

RIPA samples were analysed by discontinuous gel electrophoresis as described previously (Laemmli, 1970). The separation gel consisted of a 12 per cent (w/v) acrylamide and 0.32 per cent (w/v) N, N-methylene bis acrylamide. The samples were electrophoresed at 50 mA for 4 h or 7 mA for 18 h in Tris-glycine buffer containing 0.1% SDS. The gels were fixed in a solution of acetic acid, water and methanol in the ratio of 10:40:50 and stained in 0.1% Coomassie Blue and dried under vacuum at 80°C for 2 h. Autoradiography was carried out as described in section N.

X. Enzyme immunoassays (EIA)

Cell lysate antigen was prepared from MV-infected Vero cells. Infection of Vero cells was performed as described earlier (section H). When the cell monolayer was completely destroyed, the cells were harvested, washed and suspended in PBS, and homogenized in a Sorvall R Omnimixer. The resulting material was centrifuged once at 600 x g followed by a second centrifugation at 80,000 x g for 30 minutes. The pellet was suspended in PBS and stored at -70°C. The cell lysate antigen was sonicated prior to use.

Cell lysate antigen at a concentration of 2 µg/well was used to coat microtiter plates (Linbro, Flow laboratories, Missisauga, Ontario) in a total volume of 100 µL of PBS/well. Following overnight incubation at 4°C, 200 µL of EIA diluent (PBS supplemented with 0.5% bovine serum albumin (BSA), 0.5% Tween 20 and 0.1 mM merthiolate) was added per well, and incubated for 60 to 90 minutes at 37°C. Samples to be tested and cell lysate antigen, used as standards, were diluted in EIA diluent. Four-fold dilutions of samples and standards were mixed with fixed amounts of the different monoclonal antibodies giving an OD reading of about 1.5 in EIA binding test. The antibody-antigen mixtures were incubated overnight at 37°C. One hundred µL of the resulting mixtures were added to the EIA plates prepared as described earlier in this section and the plates were incubation at 37°C for 1 h. The cells were then washed twice with PBS supplemented with 1% Tween 20. Goat anti-mouse immunoglobulin conjugated with horse radish peroxidase was then added at a dilution of 1: 40,000 (a predetermined optimal dilution) and incubated for 1 h. The wells were washed six times. One hundred µL of substrate solution (30 mg O-phenylenediamine and 7 µL of 30% hydrogen peroxide in 10 mL of 0.1 M citrate buffer, pH 5.5) was added to each well. The plates were incubated at room temperature for 30 minutes in the dark. One hundred µL of 1 N HCl was added per well to stop the reaction and the optical density at 492 nm was read with a Titertek Multiskan photometer (Eflab, Helsinki, Finland).

Results

A. Replication of MV ts38 in PBMC

1. Release of infectious virus

MV ts38 is a ts mutant that has been isolated in Vero cells (Chui *et al.*, 1986). To determine if MV ts38 retained the ts characteristics in PBMC, comparative studies using the mutant and its parental counterpart, MV Lec was carried out in PBMC and Vero cells. First, the amount of infectious virus released at three different temperatures, 32°C, 37°C and 39°C was determined. At all three temperatures tested, more than 10^4 infectious particles per mL were detected in the SN and cells of MV Lec-infected Vero cells by day 2 (Figure 1). In contrast, in MV ts38-infected Vero cells, the amount of infectious virus detected, in SN and cells, at 37°C and 39°C was markedly decreased compared to the amount detected at 32°C (Figure 2), In MV ts38-infected Vero cells at 32°C, the cell monolayer was destroyed with large numbers of free floating cells by day 3. On the other hand, the cell monolayer was still intact at 37°C and 39°C, by day 3. The marked decrease in infectious virus detected at 37°C and 39°C in MV ts38-infected Vero cells compared to 32°C indicate that MV ts38 is a ts mutant in Vero cells, confirming previous findings (Chui *et al.*, 1986). Therefore, 32°C is the permissive temperature and 37°C as well as 39°C are restrictive temperatures.

Figure 1. Amount of infectious virus detected in supernatant solutions and cells of MV Lec-infected Vero cells grown at 32°C, 37°C or 39°C. At different times post infection, cells and supernatant solutions were harvested and tested for the presence of infectious virus by plaque assay at 37°C. The amounts of infectious virus (pfu/mL) detected are shown on a logarithmic scale.

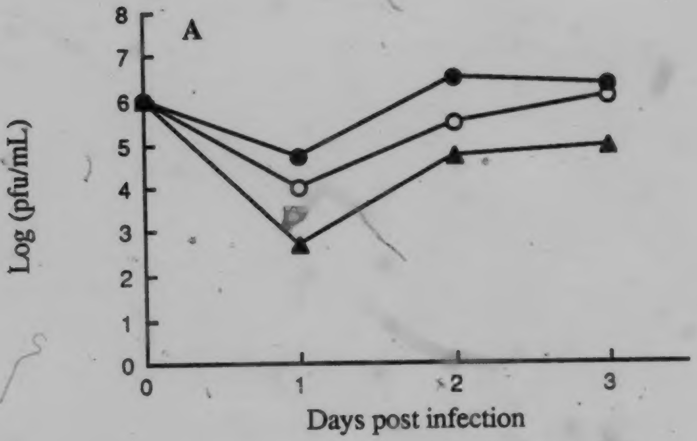
A: Amount of infectious virus detected in supernatant solutions

B: Amount of cell-associated virus detected

(○) MV Lec-infected Vero cells incubated at 32°C

(●) MV Lec-infected Vero cells incubated at 37°C

(▲) MV Lec-infected Vero cells incubated at 39°C


A
Log (pfu/mL)
8
7
6
5
4
3
2
1
0
0
1
2
3
Days post infection

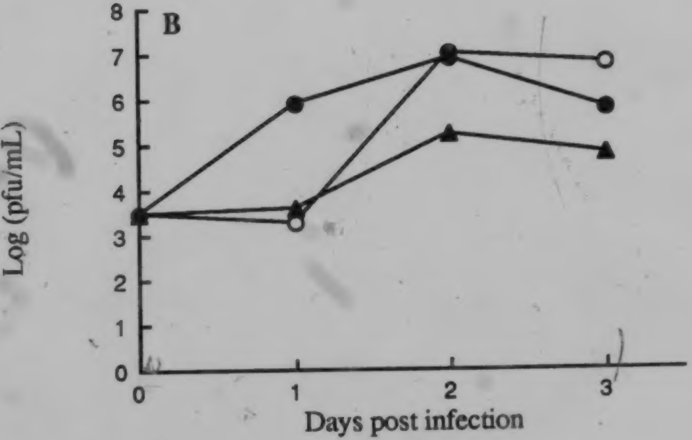

B
Log (pfu/mL)
8
7
6
5
4
3
2
1
0
0
1
2
3
Days post infection

Figure 2. Amount of infectious virus detected in supernatant solutions and cells of MV ts38-infected Vero cells grown at 32°C, 37°C and 39°C. At different times post infection, cells and supernatant solutions were harvested and tested for the presence of infectious virus by plaque assays at 32°C and 39°C. The results shown are from the plaque assays at 32°C. No plaque was obtained at 39°C. The amounts of infectious virus (pfu/mL) detected are shown on a logarithmic scale.

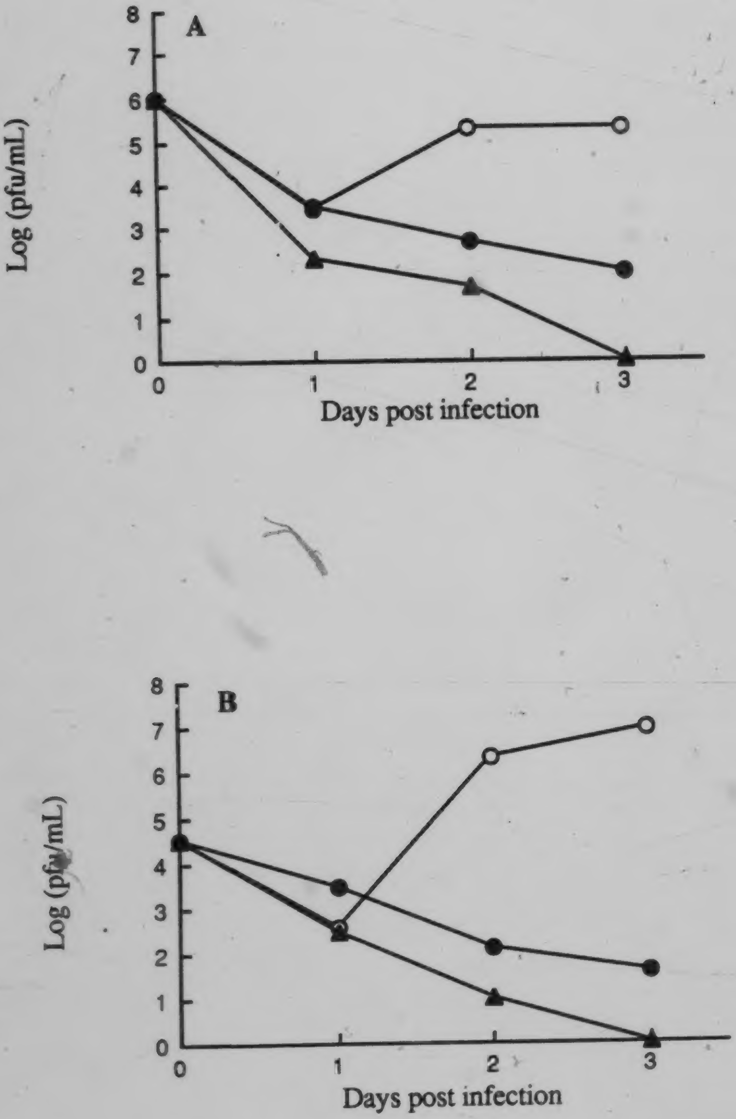
A: Amount of infectious virus detected in supernatant solutions

B: Amount of cell-associated virus detected

(○) MV ts38-infected Vero cells incubated at 32°C

(●) MV ts38-infected Vero cells incubated at 37°C

(▲) MV ts38-infected Vero cells incubated at 39°C


A
Log (pfu/mL)
8
7
6
5
4
3
2
1
0
0
1
2
3
Days post infection
B
Log (pfu/mL)
8
7
6
5
4
3
2
1
0
0
1
2
3
Days post infection

The amount of infectious virus released from PBMC infected with the parental strain is shown in Figure 3. In unstimulated PBMC at 37°C, the amount of infectious virus decreased over time resulting in a complete loss of infectivity by 30 h post infection. On the other hand, infectious particles were detected in PHA-stimulated PBMC at all three temperatures tested for longer period of times, i.e, up to 120 h at 32°C and up to 60 h at 37°C and 39°C. These results indicate that virus replication was taking place at the three temperatures in stimulated cells. The fact that infectious particles were found only for up to 20 h in unstimulated cells, with a decrease over time, indicated that no virus replication was taking place. In unstimulated PBMC, the decrease in the amount of infectious particles detected can be accounted for by the normal rate of decay of infectious particles incubated in cell-free growth medium (see Figure 5).

The amounts of infectious virus released from MV ts38-infected PBMC at different temperatures are shown in Figure 4. At 32°C, infectious virus could be detected in MV ts38-infected, PHA-stimulated PBMC for up to 120 h. At 37°C, on the other hand, infectious particles were detected only up to 30 h post infection and at 39°C, only up to 20 h. At both temperatures, the amount of infectious virus detected at any given time was lower than at the start of the experiment (0 h). A similar decrease of infectious virus over time was

Figure 3. Amount of infectious virus detected in supernatant solutions and cells of MV Lec-infected PBMC grown at 32°C, 37°C and 39°C in the presence or absence of PHA. Cells and supernatant solutions were harvested at different times and tested for the presence of infectious virus by plaque assay at 37°C. The amounts of infectious virus detected are shown on a logarithmic scale.

A: Amount of infectious virus detected in supernatant solutions

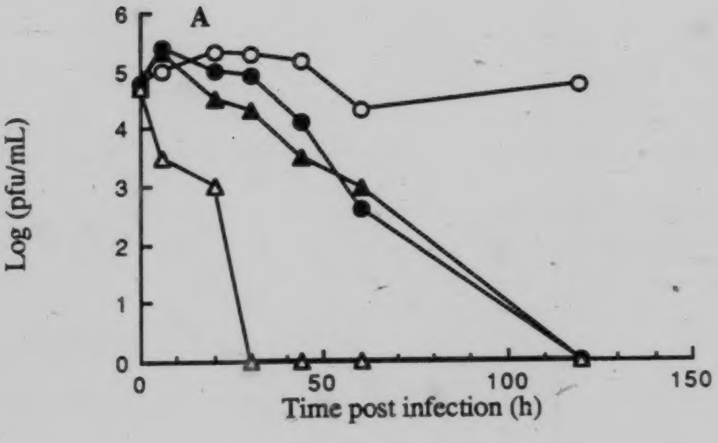
B: Amount of cell-associated virus detected

(○)MV Lec-infected and PHA-stimulated PBMC incubated at 32°C

(●)MV Lec-infected and PHA-stimulated PBMC incubated at 37°C

(▲)MV Lec-infected and PHA-stimulated PBMC incubated at 39°C

(△)Unstimulated, MV Lec-infected PBMC incubated at 37°C


A
Log (pfu/mL)
0
1
2
3
4
5
6
0
50
100
150
Time post infection (h)

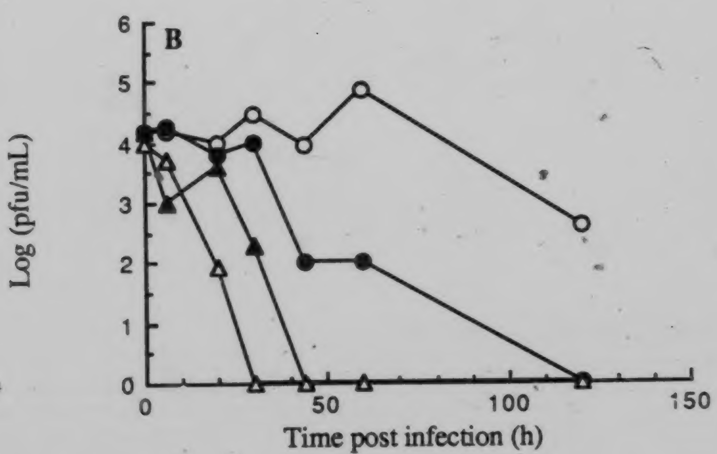

B
Log (pfu/mL)
0
1
2
3
4
5
6
0
50
100
150
Time post infection (h)

Figure 4. Amount of infectious virus detected in supernatant solutions and cells of MV ts38-infected PBMC grown at 32°C, 37°C and 39°C in the presence or absence of PHA. Cells and supernatant solutions were harvested at different times and tested for the presence of infectious virus by plaque assay at 32°C and 39°C. The results shown are from the plaque assays at 32°C. No plaque was obtained at 39°C. The amounts of infectious virus detected are shown on a logarithmic scale.

A: Amount of infectious virus detected in supernatant solutions

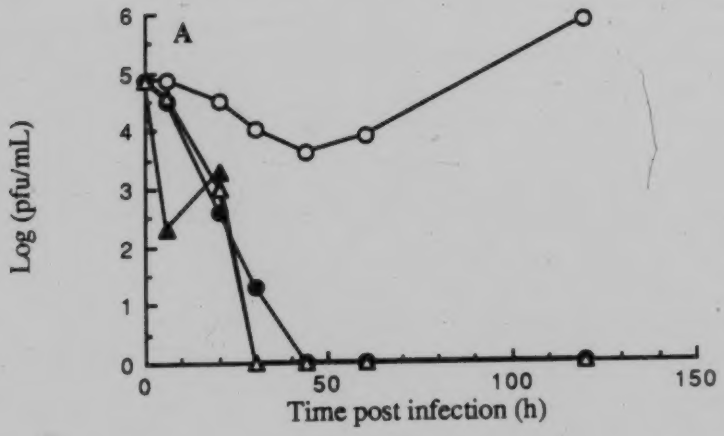
B: Amount of cell-associated virus detected

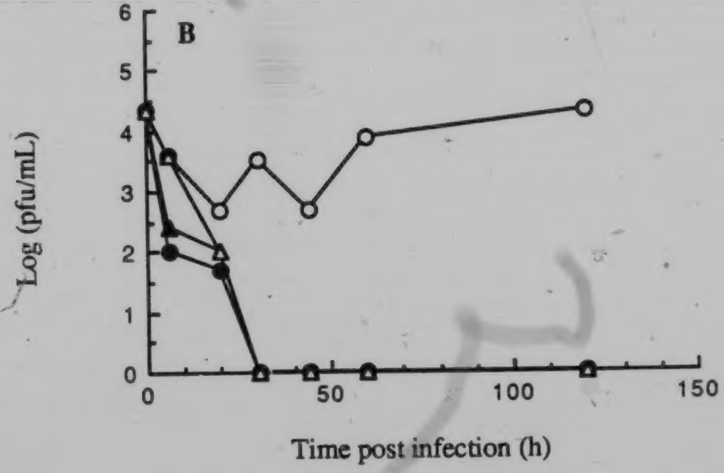
(○) MV ts38-infected and PHA-stimulated PBMC incubated at 32°C

(●) MV ts38-infected and PHA-stimulated PBMC incubated at 37°C

(▲) MV ts38-infected and PHA-stimulated PBMC incubated at 39°C

(△) Unstimulated, MV ts38-infected PBMC incubated at 37°C


A
Log (pfu/mL)
6
5
4
3
2
1
0
0
50
100
150
Time post infection (h)


B
Log (pfu/mL)
6
5
4
3
2
1
0
0
50
100
150
Time post infection (h)

observed in MV ts38-infected, unstimulated PBMC, reaching zero by 30 h. The decrease in infectivity observed in MV ts38-infected, PHA-stimulated PBMC at 37°C and 39°C followed a similar pattern to the decrease in infectious virus observed in unstimulated cells or cell-free medium (Figure 5). From these results it can be concluded that no infectious virus was being synthesized at 37°C or 39°C meaning that MV ts38 did not go through a full cycle of virus replication at 37°C or 39°C in PBMC.

Starting at 30 h post infection and any time thereafter, more than a thousand-fold difference in infectivity titer was observed when the amount of infectious virus detected at 32°C was compared to the amount detected at 37°C and 39°C, under similar conditions (Figure 4). The difference observed indicates that MV ts38 retained the ts characteristics in PBMC, with 32°C being the permissive temperature and both 37°C and 39°C being restrictive temperatures.

2. Preparation and characterization of single stranded DNA probes for the detection of MV genomic and mRNA

As MV ts38 did not go through a full cycle of virus replication at the restricted temperatures, the presence of MV mRNA was investigated. These experiments were carried out to determine at what level the replication was inhibited. As MV has a single stranded genome of (-) polarity and the mRNA

Figure 5. Rate of infectivity lost by MV ts38 and MV Lec in RPMI supplemented with 10% FBS, incubated at 32°C, 37°C and 39°C. Infectious virus (MV Lec or MV ts38) at a concentration of 10^7/mL was incubated at 32°C, 37°C and 39°C. Medium was harvested at different times and tested for infectious virus by plaque assays. For MV ts38, plaque assays were performed at 32°C and 39°C. The results shown are from plaque assays at 32°C. No plaques were obtained at 39°C. For MV Lec, plaque assays were performed at 37°C.

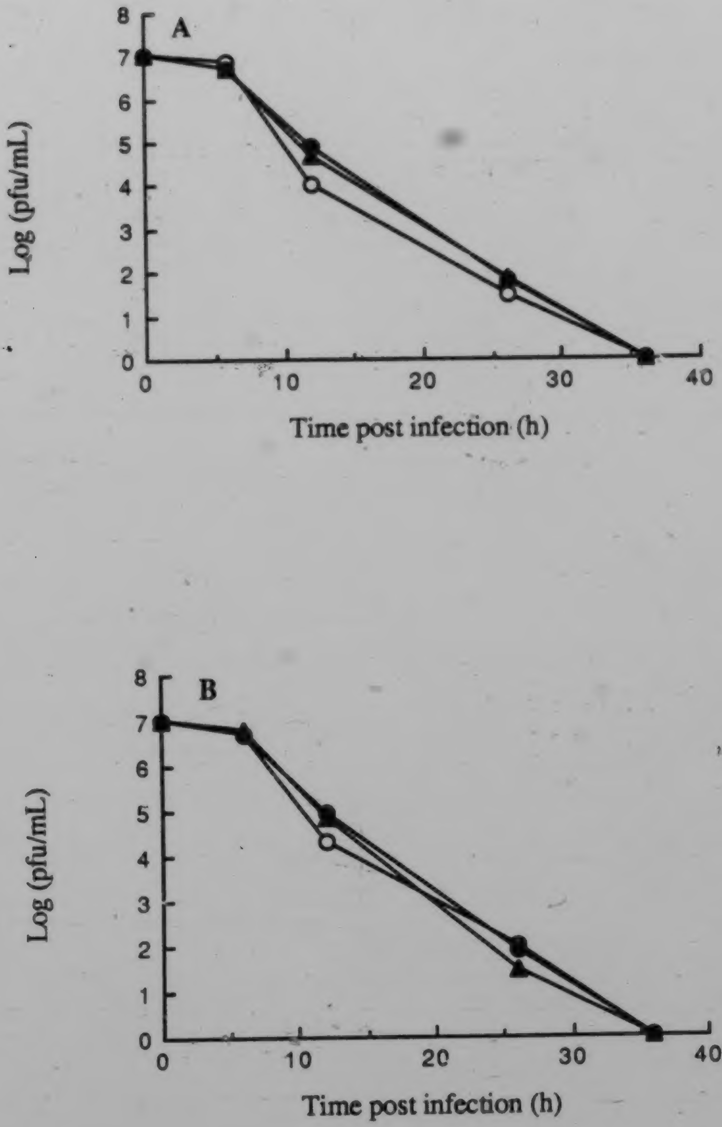
A: Rate of infectivity lost by MV ts38

B: Rate of infectivity lost by MV Lec

(○) Infectious virus incubated at 32°C

(●) Infectious virus incubated at 37°C

(▲) Infectious virus incubated at 39°C


A
B
Log (pfu/mL)
0
1
2
3
4
5
6
7
8
0
10
20
30
40
Time post infection (h)

being of (+) polarity, single stranded probes of known polarity were used in hybridization studies. For the generation of single stranded probes, ds DNA representing segments of MV genes were cloned into M13 vectors. The inserted MV DNA within the resulting single stranded clones was characterised as to its polarity before being used in hybridization studies. For that purpose, RNA from MV Edmonston-infected Vero cells was isolated by the guanidium-isothiocyanate technique. A representative sample of the RNA isolated is shown in lane C of Figure 6. Several bands were visible and two of these coincided with the 18 and 28S RNA of the ribosomal RNA standard (lane B). These results were as expected as the ribosomal RNA from the cells was in sufficiently large amounts to be seen when stained with ethidium bromide. The other bands could not be identified but were most likely cellular RNA as well. The isolated RNA was used for northern blot analysis of different M13 probes generated from MV gene fragments.

Five different MV gene fragments, N, P, M, F and H, were cloned into M13 vectors and for each gene, twelve clones were isolated. Each isolated clone was grown in suspension and DNA was isolated from the SN containing the excreted single stranded M13 phages. The isolated DNA was analysed by electrophoresis on agarose gels to determine if inserts were present. The results of a representative experiment, from clones obtained using N gene fragment, is shown in Figure 7.

Figure 6. Electrophoresis of total RNA isolated from MV Edmonston-infected Vero cells. Vero cells infected at an m.o.i. of 1, were harvested after 24 to 36 h of incubation at 37°C. Total RNA was isolated by the guanidium isothiocyanate technique and electrophoresed in a formaldehyde-agarose gel as edscribed in materials and methods. Following electrophoresis, the gel was soaked in an ethidium bromide solution followed by destaining in water. The gel was then photographed under UV light.

Lane A: RNA ladder (2 µg), units are in kilobases
Lane B: Eucaryotic ribosomal RNA (2 µg)
Lane C: Total RNA (10 µg) isolated from MV Edmonston-infected Vero cells

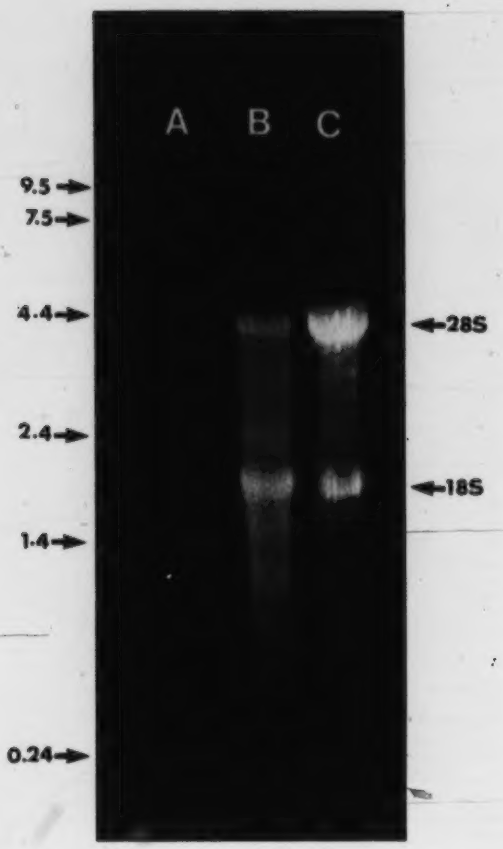

A
B
C
9.5
7.5
4.4
2.4
1.4
0.24
28S
18S

Figure 7. Electrophoresis of M13 DNA with and without inserts. M13 DNA was isolated as described in materials and methods. Different samples of isolated DNA, in a total volume of 20 µl of TE, was applied to each lane on a 1% agarose gel to which ethidium bromide had been added. Electrophoresis was for 4 to 5 h at 50 mA. The gel was then destained in water and photographed under UV light.

Lanes A and I: M13 control DNA, without insert.

The remaining lanes contain DNA isolated from M13 phages obtained from the cloning of N gene fragment into M13 DNA. Each lane represents M13 DNA obtained from one isolated clone.

A
B
C
D
E
F
G
H
I
J
K
L
M
N

Lane A and I are control lanes, containing M13 DNA without any insert; the remaining lanes contain DNA obtained from the isolated clones. The results indicated that in lanes B to G and lanes K to N, inserts were present as the rate of migration of the DNA was slower than in the control lanes. Out of the twelve clones, two were randomly selected and allowed to hybridize with the ten remaining clones. Those of opposite polarity would hybridize, resulting in a slower migration rate upon electrophoresis. A representative experiment, using isolated clones obtained from the cloning of the N gene fragment, is shown in Figure 8. The results indicated that the different combinations in lanes F, G, I, J, L, M, N, O and Q were of the same polarity as two bands were observed. The top band represented the hybrids; however, the lower band clearly indicated that not all of the DNA hybridized and those that did not, migrated to the same extent as the controls in Lane D and P. Similar experiments were carried out for each gene cloned.

Following the initial screening, two clones, each of a different polarity, were selected for each gene and analysed by northern blots to determine the polarity. The results of the northern blots analysis are shown in Figure 9. Lanes A to E showed single bands for the probes used, indicating that mRNAs were being detected. The position of the bands were also consistent with the size of the different MV mRNAs. For the N and P probes in lane A and B, bands at the 18S mark

Figure 8. Electrophoresis of M13 DNA with inserts annealed with M13 DNA containing inserts of opposite polarity. Samples, each of 20 µL, were applied to a 0.7% agarose gel containing ethidium bromide. Electrophoresis was performed for 4 to 5 h at 50 mA. Following electrophoresis, the gel was destained in water and photographed under UV light. Lanes D and P are the control lanes, with M13 DNA but without any reannealing. The remaining lanes contain isolated M13 DNA from one clone annealed with M13 DNA isolated from a different clone.

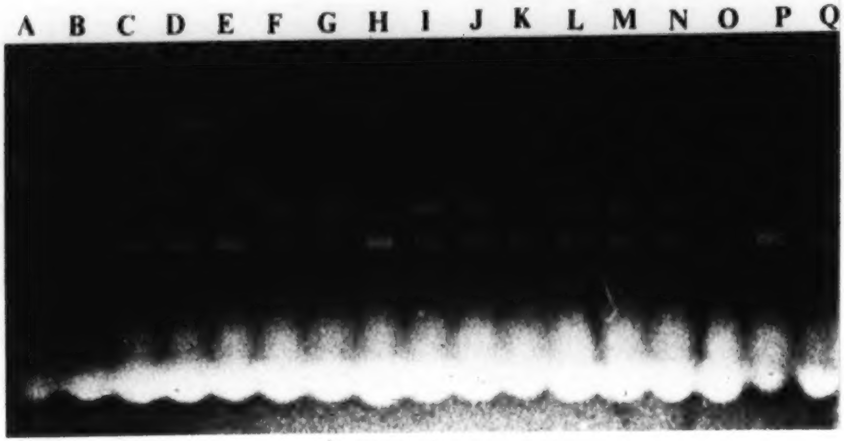
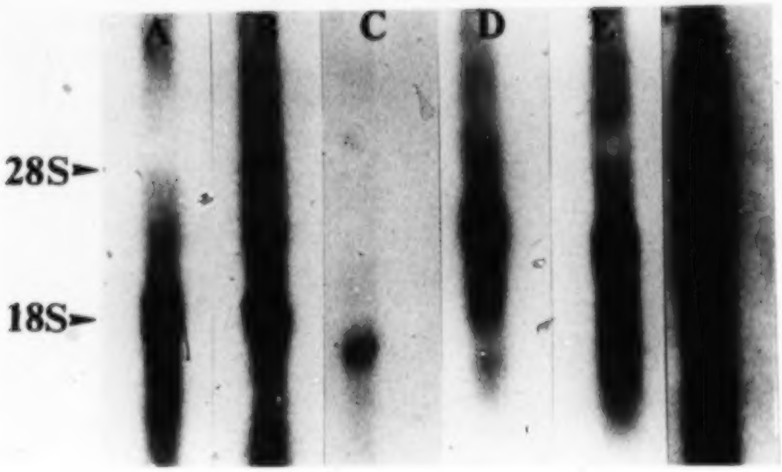

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q

Figure 9. Northern blot analysis of M13 probes. Total RNA isolated from MV Edmonston-infected Vero cells was electrophoresed, transferred onto nitrocellulose membrane and hybridized with different probes, as described in materials and methods. Following hybridization, the blots were washed and autoradiography was carried out as described in materials and methods.

Lane A: MV N probe; Lane B: MV P probe; Lane C: MV M probe; Lane D: MV F probe; Lane E: MV H probe; Lane F: MV M probe of opposite polarity to the one used in Lane C.


A
B
C
D
E
28S
18S

were obtained whereas for the M probe in lane C, a band lower than the 18S mark was detected. For both the F and H probes, lanes D and E respectively, bands obtained were in between the 18S and 28S mark, with the F band higher than that of the H. For all the genes, a smear was obtained, instead of a band when probes of opposite polarity were used (lane F). The smear represented the genomic RNA which, being relatively large, had been degraded, hence the smear. From these experiments, clones giving a distinct hybridization band at the known molecular weight of the different mRNAs, were labeled as being of negative polarity, whereas those showing a smear were designated as being of positive polarity.

3. Presence of MV mRNA and genomic RNA in infected cells

The probes were used in dot blot and northern blot experiments for the characterization of the different mRNAs. The presence of N mRNA in PBMC at 32°C and 37°C was first determined by dot blot hybridization. The results (Figure 10) indicated that mRNA of the N gene was synthesized in PBMC. However, these results, though positive, did not indicate if a full length N mRNA was synthesized. To address this question, northern blot analysis was carried out. The results (Figure 11) showed a single band at the level of the 18S RNA marker. The size of the band corresponded to the size of the N mRNA previously reported in the literature (Hasel *et al.*, 1987). No difference was observed at the three different

Figure 10. Dot blot hybridization studies. Total RNA from MV ts38-infected PBMC was isolated by a rapid method and applied to nitrocellulose membrane as described in materials and methods. Prehybridization and hybridization, using a single stranded MV N gene fragment of (-) polarity as probe, were carried out as described in materials and methods. The blots were then washed and autoradiographed. 10 µg of total RNA was used in row 1 and serial two-fold dilutions in the ensuring rows, i.e, rows 2, 3 and 4. and applies to columns A, B and C only.

A: Total RNA isolated from MV ts38-infected and PHA-stimulated PBMC, incubated at 32°C for 24 h

B: Total RNA isolated from uninfected, PHA-stimulated PBMC incubated at 37°C for 24 h

C: Total RNA isolated from MV ts38-infected and PHA-stimulated PBMC incubated at 37°C for 24 h

D1: Total RNA isolated from MV Lec-infected Vero cells (15 µg)

D2: Total RNA isolated from uninfected Vero cells (15 µg)

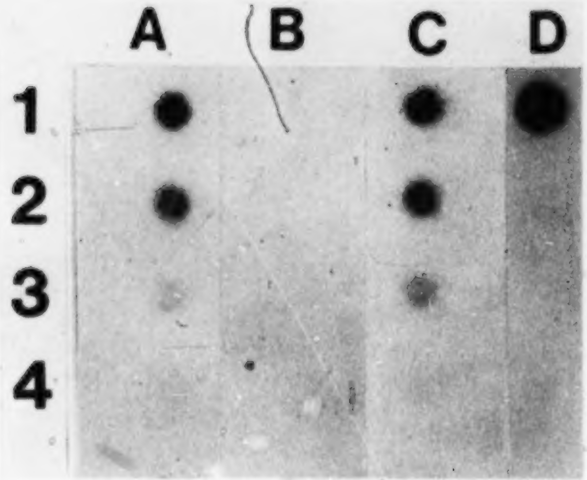

A
B
C
D
1
2
3
4

Figure 11. Detection of MV N mRNA by northern blot analysis of total RNA. Total RNA was isolated by guanidium isothiocyanate technique from MV ts38 and MV Lec-infected PBMC grown at 32°C, 37°C and 39°C. The isolated RNA was electrophoresed on a formaldehyde-agarose gel and transferred onto nylon membrane as described in materials and methods. The membrane was then prehybridized, hybridized with a single stranded MV N gene fragment of (-) polarity, washed and autoradiographed as described in materials and methods. Ten µg of total RNA was added to each lane.

Figure 11a.

Lane A: Total RNA isolated from MV ts38-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV ts38-infected PBMC incubated at 37°C for 24 to 36 h

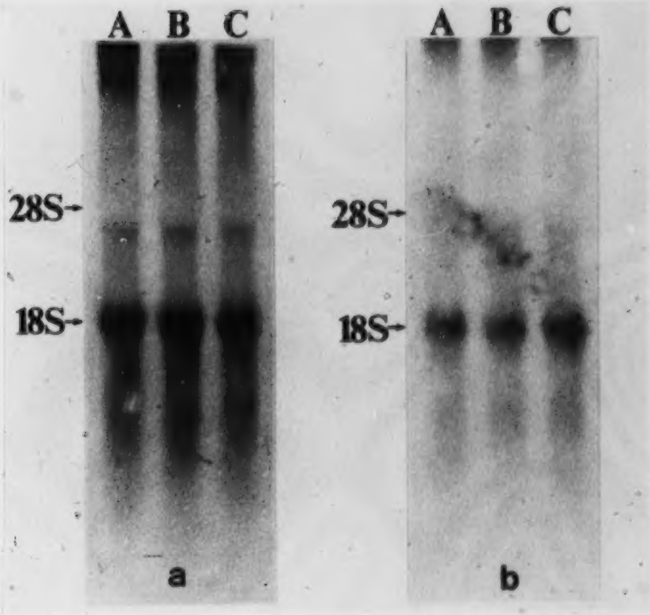
Lane C: Total RNA isolated from MV ts38-infected PBMC incubated at 39°C for 24 to 36 h

Figure 11b.

Lane A: Total RNA isolated from MV Lec-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV Lec-infected PBMC incubated at 37°C for 24 to 36 h

Lane C: Total RNA isolated from MV Lec-infected PBMC incubated at 39°C for 24 to 36 h


A
B
C
28S
18S
a
A
B
C
28S
18S
b

temperatures at which the RNA was isolated. Identical results were obtained when MV Lec-infected PBMC was examined under similar conditions except for a second band that was clearly seen in MV Lec-infected PBMC but hardly so in MV ts38-infected PBMC (Figure 11b). It most probably represented a bicistronic message of the N and P genes. The presence of a bicistronic message, though not always seen, has also been found in other MV strains (Hasel *et al.*, 1987; Yoshikawa *et al.*, 1986).

Using a probe for the M mRNA, two distinct bands were seen in both MV ts38 and MV Lec-infected PBMC (Figure 12). The lower band, smaller in size than the 18S marker, represented the M mRNA whereas the second band represented a bicistronic message corresponding to the M and F genes. It has previously been reported that three bands could be detected in MV-infected cells representing the M mRNA and two bicistronic messages namely, M-P and M-F (Wong *et al.* 1987; Yoshikawa *et al.*, 1986). The two bicistronic messages are of the same size, implying that the second band could represent two different bicistronic mRNAs.

Figures 13a and 13b show the bands obtained when probes were used to detect the H mRNA. A distinct band found above the 18S mark was obtained at all three temperatures tested, in both MV ts38 and MV Lec-infected PBMC. No clear distinct bicistronic message was detected. In summary, for all the genes tested, there was no defect in the synthesis of viral

Figure 12. Detection of MV M mRNA by northern blot analysis of total RNA. Total RNA was isolated by guanidium isothiocyanate technique from MV ts38 and MV Lec-infected PBMC grown at 32°C, 37°C and 39°C. The isolated RNA was electrophoresed on a formaldehyde-agarose gel and transferred onto nylon membrane as described in materials and methods. The membrane was then prehybridized, hybridized with a single stranded MV M gene fragment of (-) polarity, washed and autoradiographed as described in materials and methods. Ten µg of total RNA was added to each lane.

Figure 12a.

Lane A: Total RNA isolated from MV ts38-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV ts38-infected PBMC incubated at 37°C for 24 to 36 h

Lane C: Total RNA isolated from MV ts38-infected PBMC incubated at 39°C for 24 to 36 h

Figure 12b.

Lane A: Total RNA isolated from MV Lec-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV Lec-infected PBMC incubated at 37°C for 24 to 36 h

Lane C: Total RNA isolated from MV Lec-infected PBMC incubated at 39°C for 24 to 36 h

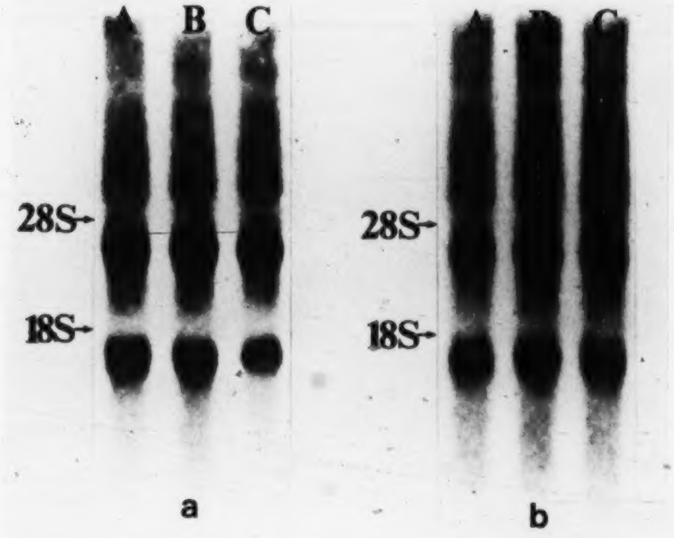

A
B
C
28S
18S
a
A
B
C
28S
18S
b

Figure 13. Detection of MV H mRNA by northern blot analysis of total RNA. Total RNA was isolated by guanidium isothiocyanate technique from MV ts38 and MV Lec-infected PBMC grown at 32°C, 37°C and 39°C. The isolated RNA was electrophoresed on a formaldehyde-agarose gel and transferred onto nylon membrane as described in materials and methods. The membrane was then prehybridized, hybridized with a single stranded MV H gene fragment of (-) polarity, washed and autoradiographed as described in materials and methods. Ten µg of total RNA was added to each lane.

Figure 13a.

Lane A: Total RNA isolated from MV ts38-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV ts38-infected PBMC incubated at 37°C for 24 to 36 h

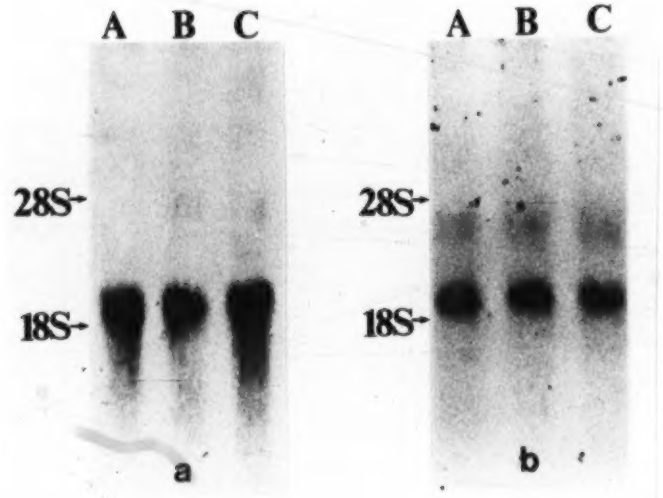
Lane C: Total RNA isolated from MV ts38-infected PBMC incubated at 39°C for 24 to 36 h

Figure 13b.

Lane A: Total RNA isolated from MV Lec-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV Lec-infected PBMC incubated at 37°C for 24 to 36 h

Lane C: Total RNA isolated from MV Lec-infected PBMC incubated at 39°C for 24 to 36 h


A
B
C
28S
18S
a
A
B
C
28S
18S
b

mRNA in MV ts38-infected PBMC as similar size messages were detected at both the permissive and the restricted temperatures. In addition, all the mRNAs detected corresponded in size to those obtained from the parental strain.

The end result of MV replication is the accumulation of the viral genome within the cell prior to assembly. To determine if MV genome was being synthesized within MV ts38-infected PBMC at the different temperatures, a probe of positive polarity derived from the M gene was used. The hybridization results showed only one large band at the top of the blot (Figure 14b). Similar results were obtained for MV Lec-infected PBMC (Figure 14a). These results indicated that an RNA species of high molecular weight was present at both the restrictive as well as the permissive temperatures. The relative size of the RNA detected and the polarity suggested that it represented the genomic RNA. The results also confirmed the specificity of the probes, as no other band was detected. The pattern of MV ts38 replication in PBMC that emerged from Figures 10 to 14 was as follows:

1. No difference was observed at 32°C, compared to 37°C and 39°C, in MV ts38-infected PBMC with respect to transcription.

2. No difference was observed in mRNA size between MV ts38 and MV Lec-infected PBMC at any of the temperatures tested.

Figure 14. Detection of MV genomic RNA by northern blot analysis of total RNA. Total RNA was isolated by guanidium isothiocyanate technique from MV ts38 and MV Lec-infected PBMC grown at 32°C, 37°C and 39°C. The isolated RNA was electrophoresed on a formaldehyde-agarose gel and transferred onto nylon membrane as described in materials and methods. The membrane was then prehybridized, hybridized with a single stranded MV M gene fragment of (+) polarity, washed and autoradiographed as described in materials and methods. Ten µg of total RNA was added to each lane.

Figure 14a.

Lane A: Total RNA isolated from MV ts38-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV ts38-infected PBMC incubated at 37°C for 24 to 36 h

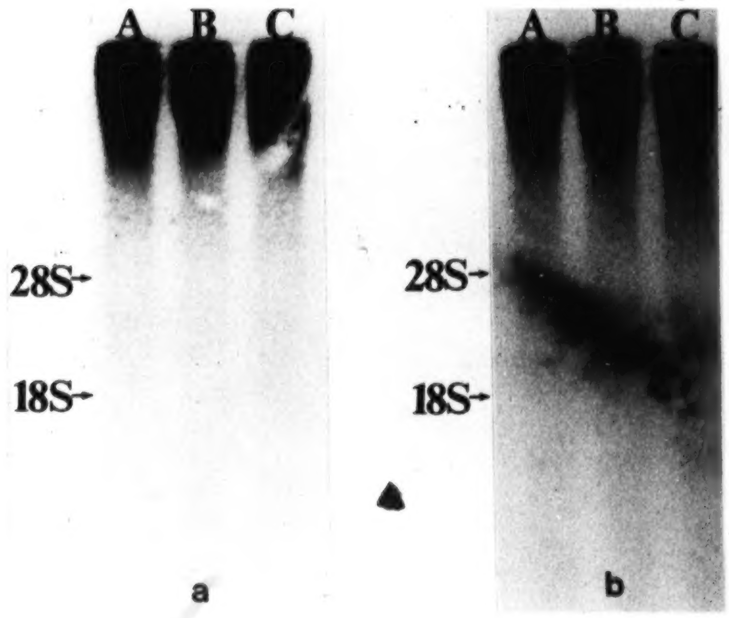
Lane C: Total RNA isolated from MV ts38-infected PBMC incubated at 39°C for 24 to 36 h

Figure 14b.

Lane A: Total RNA isolated from MV Lec-infected PBMC incubated at 32°C for 24 to 36 h

Lane B: Total RNA isolated from MV Lec-infected PBMC incubated at 37°C for 24 to 36 h

Lane C: Total RNA isolated from MV Lec-infected PBMC incubated at 39°C for 24 to 36 h


A
B
C
28S
18S
a
A
B
C
28S
18S
b

3. (-) strand RNA of genomic size was present at the different temperatures tested.

Similar experiments were conducted in MV ts38 and MV Lec-infected Vero cells. Preliminary experiments had indicated that mRNA was present at 32°C only by day 2. Thus more extensive experiments were conducted. RNA from MV ts38 and MV Lec-infected Vero cells was isolated at days 2, 4 and 6 post infection and northern blot analysis was performed. Using a probe for N mRNA, a distinct band migrating at the 18S level was seen in MV Lec-infected Vero cells at all temperatures tested at days 2, 4 and 6 (Figure 15b). The band most likely represented the N mRNA. A second band, representing a bicistronic message, N-P, was also seen in the region between the 18 and 28S region. In MV ts38-infected Vero cells, on the other hand, mRNA for the N gene was detected for the three days tested, at 32°C only (Figure 15a). At 37°C, detectable levels of the N gene mRNA was obtained only by day 4 and at 39°C, by day 6 only. The size of the message at all three temperatures tested was similar.

H mRNA was detected at all times at 32°C, 37°C and 39°C in MV Lec-infected Vero cells (Figure 16a) whereas in MV ts38-infected Vero cells, the message was detected at all times tested at 32°C, by day 4 at 37°C and day 6 at 39°C (Figure 16b).

The presence of viral mRNA at 4 days post infection at 37°C and at 6 days at 39°C indicate that the rate of

Figure 15. Time course analysis of MV N mRNA synthesis by northern blot. Total RNA was isolated by guanidium isothiocyanate technique from MV ts38 and MV Lec-infected Vero cells grown at 32°C, 37°C and 39°C. The isolated RNA was electrophoresed on a formaldehyde-agarose gel and transferred onto nylon membrane as described in materials and methods. The membrane was then prehybridized, hybridized with a single stranded MV N gene fragment of (-) polarity, washed and autoradiographed as described in materials and methods. Ten µg of total RNA was added to each lane.

Figure 15a

Lanes A, B and C: Total RNA isolated from MV ts38-infected Vero cells incubated at 32°C for 2, 4 and 6 days respectively. Lanes D, E and F: Total RNA isolated from MV ts38-infected Vero cells incubated at 37°C for 2, 4 and 6 days respectively. Lanes G, H and I: Total RNA isolated from MV ts38-infected Vero cells incubated at 39°C for 2, 4 and 6 days respectively.

Figure 15b

Lanes A, B and C: Total RNA isolated from MV Lec-infected Vero cells incubated at 32°C for 2, 4 and 6 days respectively. Lanes D, E and F: Total RNA isolated from MV Lec-infected Vero cells incubated at 37°C for 2, 4 and 6 days respectively. Lanes G, H and I: Total RNA isolated from MV Lec-infected Vero cells incubated at 39°C for 2, 4 and 6 days respectively.

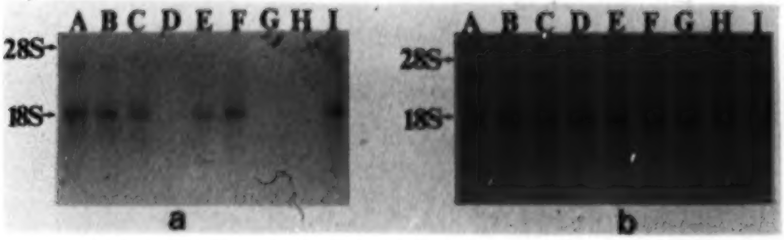

A B C D E F G H I
28S
18S
a
A B C D E F G H I
28S
18S
b

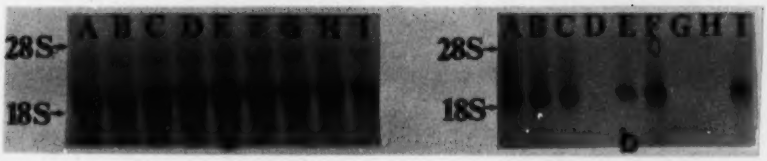
Figure 16. Time course analysis of MV H mRNA synthesis by northern blot. Total RNA was isolated by guanidium isothiocyanate technique from MV ts38 and MV Lec-infected Vero cells grown at 32°C, 37°C and 39°C. The isolated RNA was electrophoresed on a formaldehyde-agarose gel and transferred onto nylon membrane as described in materials and methods. The membrane was then prehybridized, hybridized with a single stranded MV H gene fragment of (-) polarity, washed and autoradiographed as described in materials and methods. Ten µg of total RNA was added to each lane.

Figure 16a

Lanes A, B and C: Total RNA isolated from MV Lec-infected Vero cells incubated at 32°C for 2, 4 and 6 days respectively. Lanes D, E and F: Total RNA isolated from MV Lec-infected Vero cells incubated at 37°C for 2, 4 and 6 days respectively. Lanes G, H and I: Total RNA isolated from MV Lec-infected Vero cells incubated at 39°C for 2, 4 and 6 days respectively.

Figure 16b

Lanes A, B and C: Total RNA isolated from MV ts38-infected Vero cells incubated at 32°C for 2, 4 and 6 days respectively. Lanes D, E and F: Total RNA isolated from MV ts38-infected Vero cells incubated at 37°C for 2, 4 and 6 days respectively. Lanes G, H and I: Total RNA isolated from MV ts38-infected Vero cells incubated at 39°C for 2, 4 and 6 days respectively.


28S
18S
A B C D E F G H I
28S
18S
A B C D E F G H I

transcription decreases with temperature. mRNA synthesis was at a much lower rate at the restrictive temperatures compared to the permissive temperature, reaching detectable levels by day 4 post infection at 37°C, and by day 6 at 39°C. These results suggest that the ts properties of MV ts38 in Vero cells might be due to the reduced level of transcriptional activity at the restricted temperatures.

4. Viral protein synthesis in infected cells

The next step in MV replication, following mRNA synthesis is the translation of the message resulting in protein synthesis. To determine if viral proteins were being synthesized, immunofluorescence studies, using monoclonal antibodies against different viral proteins were carried out. Using monoclonal antibodies against N protein, MV Lec-infected Vero cells were compared to MV ts38-infected Vero cells at 32°C, 37°C and 39°C. These experiments were conducted at days 1, 2 and 3 post infection. The results, in Figure 17 showed the presence, at day 1, of N protein at all three temperatures tested for MV Lec-infected Vero cells. However, N protein was detected at 32°C in MV ts38-infected Vero cells but not at 37°C or 39°C. Similar results were obtained for days 2 and 3 post infection. On the other hand, N protein was being synthesized at all three temperatures tested in MV ts38-infected PBMC. These results are shown in Figure 18. Using monoclonal antibodies against P, similar experiments as for N were carried out and the same pattern

Figure 17. Immunofluorescence studies of MV Lec-infected Vero cells and MV ts38-infected Vero cells at 32°C, 37°C and 39°C using monoclonal antibodies against MV N protein. Vero cells were infected with either MV ts38 or MV Lec as described in materials and methods and incubated for 24 h. The cells were then harvested, fixed in acetone at -20°C and stained with monoclonal antibodies against N protein at a dilution of 1:50 followed by goat anti-mouse FITC at a dilution of 1:100, as described in materials and methods. Cells were then washed and photographed (Magnification: 400x).

A, B and C: MV Lec-infected Vero cells grown at 32°C, 37°C and 39°C respectively.

D, E and F: MV ts38-infected Vero cells grown at 32°C, 37°C and 39°C respectively.

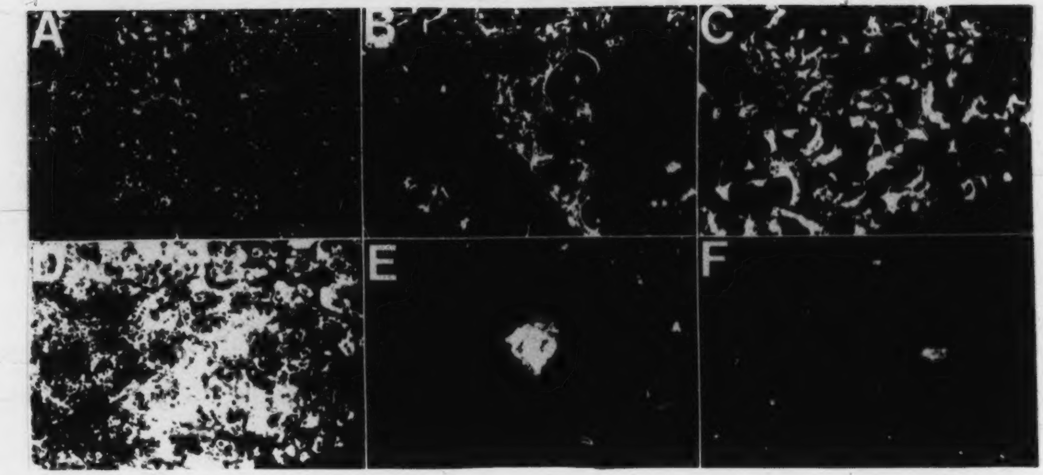
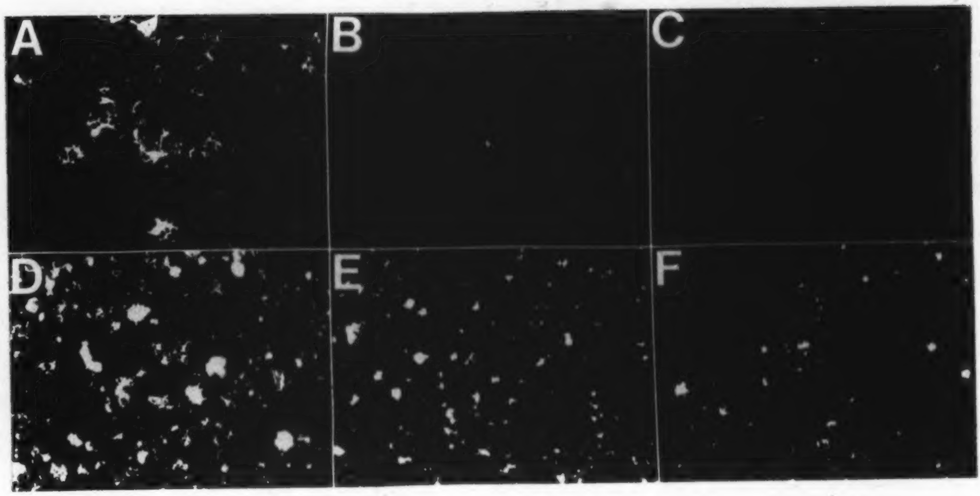

A
B
C
D
E
F

Figure 18. Immunofluorescence studies of MV ts38-infected Vero cells and PBMC at 32°C, 37°C and 39°C, using monoclonal antibodies against MV N protein. Vero cells and PBMC were infected with MV ts38 as described in materials and methods and incubated for 24 h. The cells were then harvested, fixed in acetone at -20°C and stained with monoclonal antibodies against N protein at a dilution of 1:50 followed by goat anti-mouse FITC at a dilution of 1:100, as described in materials and methods. Cells were then washed an photographed (Magnification: 400x).

A, B and C: MV ts38-infected Vero cells grown at 32°C, 37°C and 39°C respectively.

D, E and F: MV ts38-infected PBMC grown at 32°C, 37°C and 39°C respectively.


A
B
C
D
E
F

was observed (Figure 19). There was no P protein in infected Vero cells at the restricted temperatures whereas P protein was clearly visible at both the permissive and restrictive temperatures in infected PBMC.

Similar observations were made when monoclonal antibodies against M, F and H were used (Figures 20 to 22). These results indicated that all viral proteins were being synthesized at both the permissive and restrictive temperatures in MV ts38-infected PBMC, whereas there was no viral protein synthesis at the restrictive temperatures in MV ts38-infected Vero cells. Results from the immunofluorescence studies indicated that viral proteins were being synthesized in MV ts38-infected PBMC. However, the results did not indicate whether the viral proteins synthesized were full size proteins or truncated versions. The sizes of the different viral proteins were examined by radioimmunoprecipitation followed by electrophoresis on polyacrylamide gels. The results showed that at all temperatures tested, i.e, 32°C, 37°C and 39°C, full size viral proteins were being synthesized (Figure 23). Several bands were found in MV-infected PBMC and not in uninfected PBMC. Six of these bands were identified as full size viral proteins based on their rate of migration. The bands identified were H, P, N, A for actin, F_1 and M. No difference in the size of the different viral proteins was detected in MV ts38-infected PBMC (Figure 23a) when compared to MV Lec-

Figure 19. Immunofluorescence studies of MV ts38-infected Vero cells and PBMC at 32°C, 37°C and 39°C, using monoclonal antibodies against MV P protein. Vero cells and PBMC were infected with MV ts38 as described in materials and methods and incubated for 24 h. The cells were then harvested, fixed in acetone at -20°C and stained with monoclonal antibodies against P protein at a dilution of 1:50 followed by goat anti-mouse FITC at a dilution of 1:100, as described in materials and methods. Cells were then washed and photographed (Magnification: 400x).

A, B and C: MV ts38-infected Vero cells grown at 32°C, 37°C and 39°C respectively.

D, E and F: MV ts38-infected PBMC grown at 32°C, 37°C and 39°C respectively.

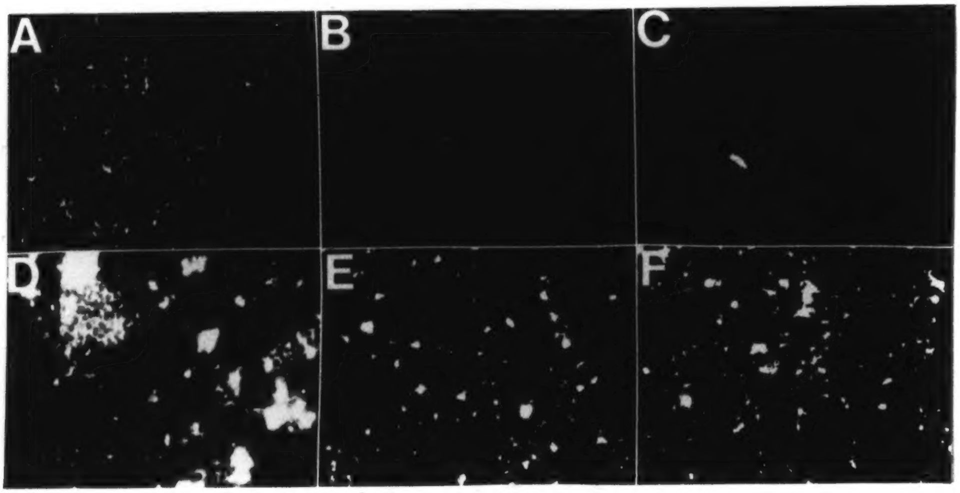

A
B
C
D
E
F

Figure 20. Immunofluorescence studies of MV ts38-infected Vero cells and PBMC at 32°C, 37°C and 39°C, using monoclonal antibodies against MV M protein. Vero cells and PBMC were infected with MV ts38 as described in materials and methods and incubated for 24 h. The cells were then harvested, fixed in acetone at -20°C and stained with monoclonal antibodies against M protein at a dilution of 1:50 followed by goat anti-mouse FITC at a dilution of 1:100, as described in materials and methods. Cells were then washed and photographed (Magnification: 400x).

A, B and C: MV ts38-infected Vero cells grown at 32°C, 37°C and 39°C respectively.

D, E and F: MV ts38-infected PBMC grown at 32°C, 37°C and 39°C respectively.

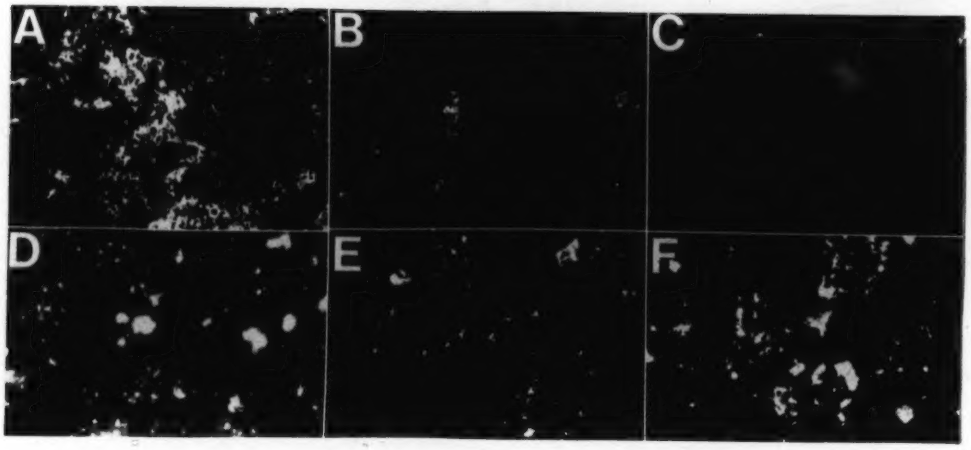

A
B
C
D
E
F

Figure 21. Immunofluorescence studies of MV ts38-infected Vero cells and PBMC at 32°C, 37°C and 39°C, using monoclonal antibodies against MV F protein. Vero cells and PBMC were infected with MV ts38 as described in materials and methods and incubated for 24 h. The cells were then harvested, fixed in acetone at -20°C and stained with monoclonal antibodies against F protein at a dilution of 1:50 followed by goat anti-mouse FITC at a dilution of 1:100, as described in materials and methods. Cells were then washed and photographed (Magnification: 400x).

A, B and C: MV ts38-infected Vero cells grown at 32°C, 37°C and 39°C respectively.

D, E and F: MV ts38-infected PBMC grown at 32°C, 37°C and 39°C respectively.

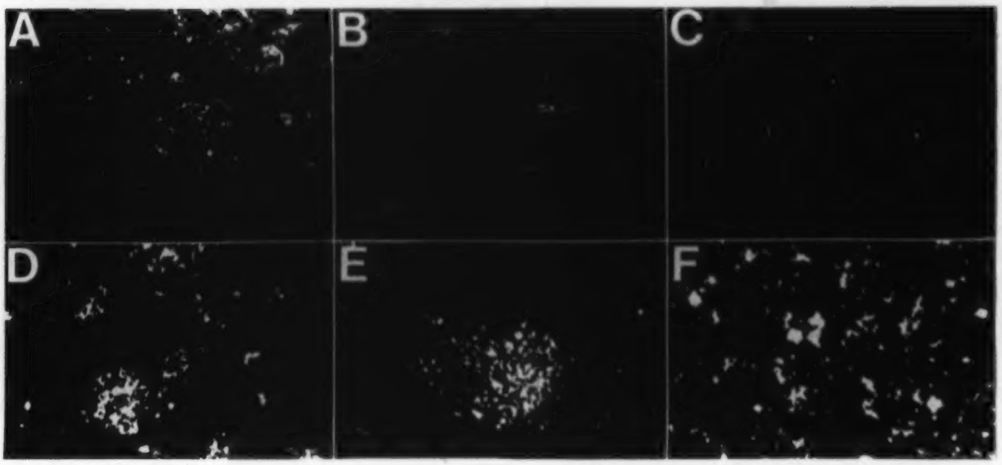

A
B
C
D
E
F

Figure 22. Immunofluorescence studies of MV ts38-infected Vero cells and PBMC at 32°C, 37°C and 39°C, using monoclonal antibodies against MV H protein. Vero cells and PBMC were infected with MV ts38 as described in materials and methods and incubated for 24 h. The cells were then harvested, fixed in acetone at -20°C and stained with monoclonal antibodies against H protein at a dilution of 1:50 followed by goat anti-mouse FITC at a dilution of 1:100, as described in materials and methods. Cells were then washed and photographed (Magnification: 400x).

A, B and C: MV ts38-infected Vero cells grown at 32°C, 37°C and 39°C respectively.

D, E and F: MV ts38-infected PBMC grown at 32°C, 37°C and 39°C respectively.

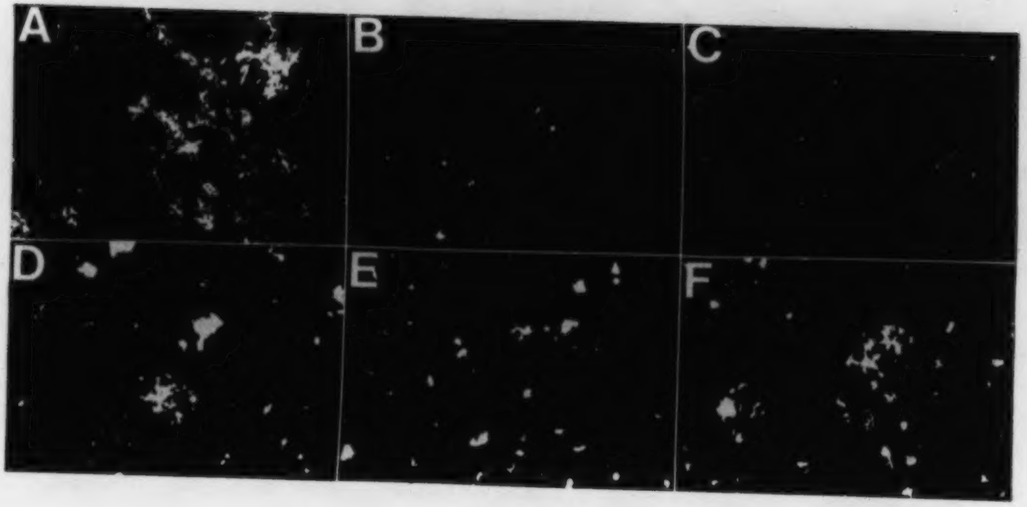

A
B
C
D
E
F

Figure 23. Presence of MV proteins at 32°C, 37°C and 39°C in MV ts38 and MV Lec-infected PBMC. PBMC were infected at an m.o.i. of 3 to 5 and grown at 32°C, 37°C or 39°C in medium containing [^{35}S]-methionine and 10 µg/mL of PHA, as described in materials and methods. The cells were harvested 32 to 34 h later. Viral proteins were immunoprecipitated with anti-MV polyclonal serum and electrophoresed in polyacrylamide gels. The gels were then dried under vacuum and autoradiographed.

Figure 23a.

A: Viral proteins from MV ts38-infected PBMC incubated at 32°C

B: Viral proteins from MV ts38-infected PBMC incubated at 37°C

C: Viral proteins from MV ts38-infected PBMC incubated at 39°C.

D: Uninfected PBMC incubated at 32°C

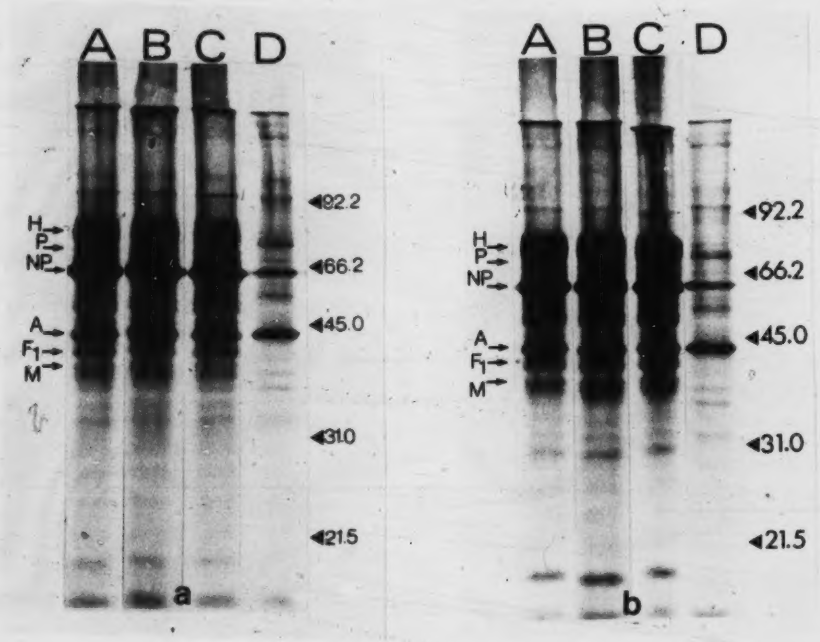
Figure 23b.

A: Viral proteins from MV Lec-infected PBMC incubated at 32°C

B: Viral proteins from MV Lec-infected PBMC incubated at 37°C

C: Viral proteins from MV Lec-infected PBMC incubated at 39°C.

D: Uninfected PBMC incubated at 32°C


A
B
C
D
H
P
NP
A
F1
M
92.2
66.2
45.0
31.0
21.5
a
A
B
C
D
H
P
NP
A
F1
M
92.2
66.2
45.0
31.0
21.5
b

infected PBMC (Figure 23b) at any of the three temperatures tested. The presence of F_1 indicated that the F protein was being cleaved. However, F_2, the other product of F cleavage, was not detected. This is because F_2 contains very few methionine residues and is not seen by [^{35}S]-methionine labeling (Shesbaradaran *et al.*, 1985).

The amounts of two different viral proteins, namely N and H, in MV ts38-infected PBMC, were determined by indirect enzyme immunoassays (EIA) as described in materials and methods. The amounts of viral proteins detected in PBMC were expressed as a percentage of the amount of viral antigens present in MV Edmonston-infected Vero cells, prepared as described in materials and methods. The results (Table 2) indicated that no significant difference was observed when the amounts of viral proteins detected at the permissive temperature were compared with the amounts detected at the restrictive temperatures. However, a significant difference was observed when the amount of N was compared with the amount of H detected at any one temperature. At all temperatures tested, two to three times more N than H was detected. These results are in agreement with what has been published, i.e, the nucleocapsid protein is the most abundant one in the virion as well as in infected cells (Rima *et al.*, 1982)

Table 2. Amount of different viral proteins in MV ts38-infected PBMC grown at different temperatures at 24 h post infection.

		% of MV proteins present	
Cell type	Temperature	N	H
PBMC	32°C	48±4	16±2
	37°C	36±6	21±1.8
	39°C	39±8	17±2.1

EIA were performed as described in materials and methods. Cell lysate antigen was used to standardize the system. A standard curve was drawn from OD results obtained when different dilutions of cell lysate antigen were used. MV ts38-infected PBMC samples were tested in a 1:2 dilution. The OD results obtained from the samples were compared to the standard curve. The results are expressed as the percentage of MV protein present in cell lysate antigen at a dilution of 1:50. Mean and standard deviations were calculated from triplicate wells.

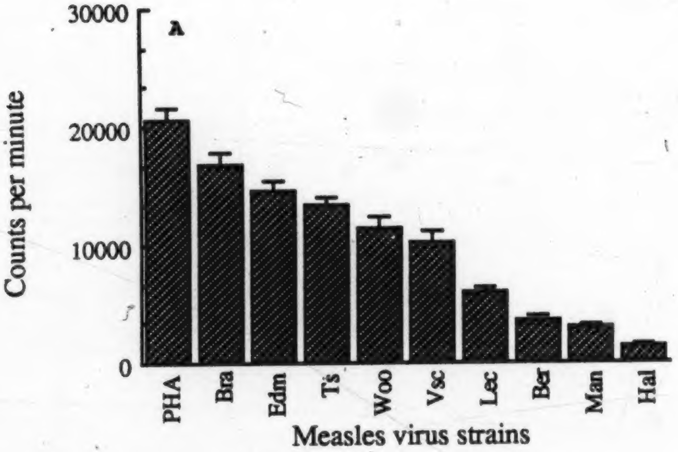
B. Effect of MV infection on PBMC responses to various stimuli

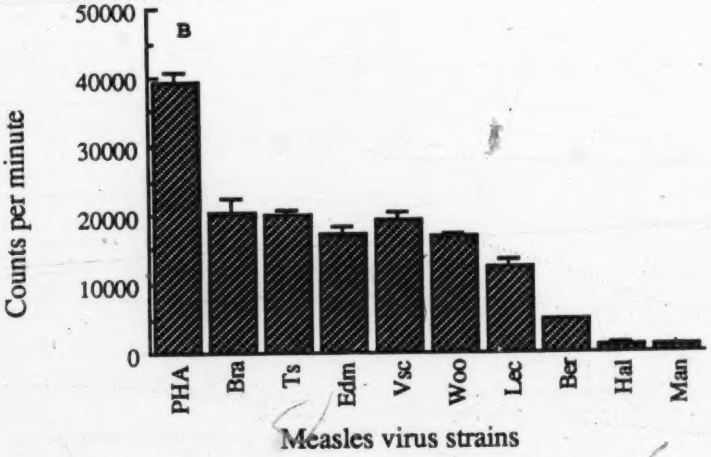
The results presented so far indicate that MV ts38 retained the temperature sensitivity in PBMC at 37°C. In so doing, the virus induces an abortive infection, i.e, the virus does not undergo a full cycle of replication. Thus, experiments were designed to determine the effect of MV infection, under abortive conditions, on the response of PBMC to various stimuli. As it had previously been shown that the effect of MV on PBMC proliferation varied depending upon the strain used (Ilonen *et al.*, 1988), the effect of MV ts38 on PBMC proliferation was compared to that of the parental strain as well as seven other strains. Substantial differences in the inhibitory effect of the different strains were observed, ranging from 50 to greater than 90% by day 3 depending upon the strain used (Figure 24b). The inhibitory effect was observed at both days tested, and was more pronounced by day 3 for every strain used (Figure 24a and 24b). MV ts38 blocked the proliferative response of PBMC by 50%, similar to MV Bray, MV VSC (two wild type isolates), and MV Edmonston, a vaccine strain; the parental strain together with MV Halle, MV Berg, and MV Mantooth, all SSPE isolates, were more inhibitory to the proliferative response of PBMC to PHA.

Figure 24. Effect of different strains of MV on the proliferation of PBMC stimulated by PHA. PBMC were infected at an m.o.i. of 5 and stimulated with 10 µg/mL of PHA. [^{3}H]- thymidine was added for the last 24 h of a total incubation period of either 48 or 72 h at 37°C. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). Virus strains used were as follows: MV Bray (Bra); MV Edmonston (Edm); MV ts38 (Ts); MV Woodfolk (Woo); MV Lec (Lec); MV Berg (Ber); MV Mantooth (Man) and MV Halle (Hal); PHA-stimulated and uninfected cultures (PHA). Each column represents the mean and standard deviation of six parallel cultures.

A: PBMC were incubated for 48 h before harvest

B: PBMC were incubated for 72 h before harvest


A
Counts per minute
30000
20000
10000
0
PHA
Bra
Edm
Ts
Woo
Vsc
Lec
Ber
Man
Hal
Measles virus strains


B
Counts per minute
50000
40000
30000
20000
10000
0
PHA
Bra
Ts
Edm
Vsc
Woo
Lec
Ber
Hal
Man
Measles virus strains

Antibodies directed against the T cell marker, OKT3, were also used as stimulus for PBMC in the presence or absence of MV ts38 and MV Lec. The amount of tritiated thymidine incorporated was lower than when PHA was used to stimulate the cells. However, both MV Lec and MV ts38 blocked the proliferative response as shown in Figure 25, without any significant difference detected between the two strains. The effects of MV infection on a one-way and a two-way mixed lymphocyte reaction are shown in Figure 26a and b. The inhibitory effect of both MV ts38 and MV Lec was more than 80% by day 5 in a one-way mixed lymphocyte reaction in which the responder cells were infected. Similar results were obtained in two-way mixed lymphocyte reactions. In both cases, no significant difference was observed between MV ts38 and MV Lec inhibitory effect. Figure 27 showed the results of an experiment in which HSV antigens at a concentration of 10 µg/mL were used to stimulate PBMC from HSV-positive patients, in the presence or absence of MV. Both the mutant and the parental strains blocked the proliferative response induced by the antigen. These results, taken together, clearly indicated that MV interfered with PBMC proliferation *in vitro*, irrespective of the stimulus used. No significant difference was observed between the effect of MV ts38 and MV Lec except when PHA was used as a stimulus.

Figure 25. Effect of MV ts38 and MV Lec on the stimulation of PBMC by anti- OKT3 antibodies. PBMC were infected at a m.o.i. of 5 with either MV Lec or MV ts38 and stimulated with anti-OKT3 antibodies at a dilution of 1:50. Cells were incubated at 37°C for 72 h. [^{3}H]-thymidine was added for the last 18 h of incubation. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). Each column represents the mean and standard deviation of six parallel cultures.

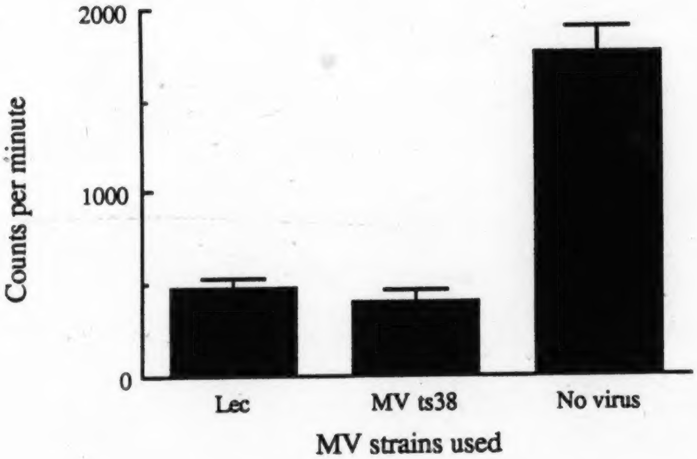

2000
1000
0
Counts per minute
Lec
MV ts38
No virus
MV strains used

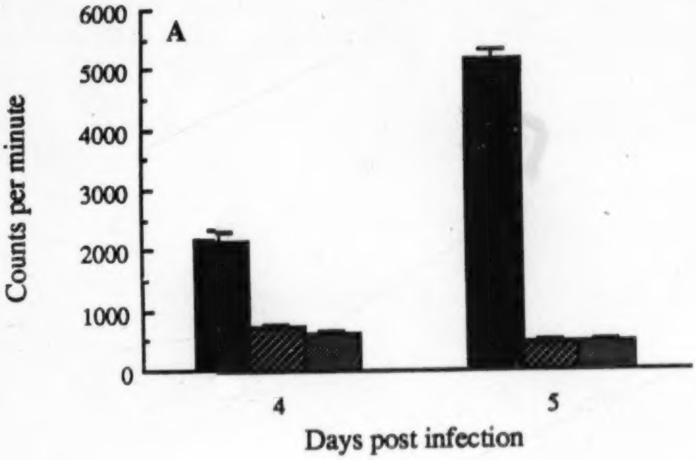
Figure 26. Effect of MV ts38 or MV Lec infection on a one-way and a two-way mixed lymphocyte reaction. PBMC were infected at a m.o.i. of 5 with either MV ts38 or MV Lec and mixed with allogeneic PBMC that were either infected with the same MV strain or uninfected and irradiated as described in materials and methods. The cells were mixed at a concentration of 10^6/mL each and incubated for either 4 or 5 days at 37°C and $[^3H]$-thymidine added for the last 18 h of incubation. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). Each column represents the mean and standard deviation of six parallel cultures.

● Uninfected PBMC

◉ MV ts38-infected PBMC

▨ MV Lec-infected PBMC

A: One-way mixed lymphocyte reaction.
B: Two-way mixed lymphocyte reaction.


A
Counts per minute
6000
5000
4000
3000
2000
1000
0
4
5
Days post infection

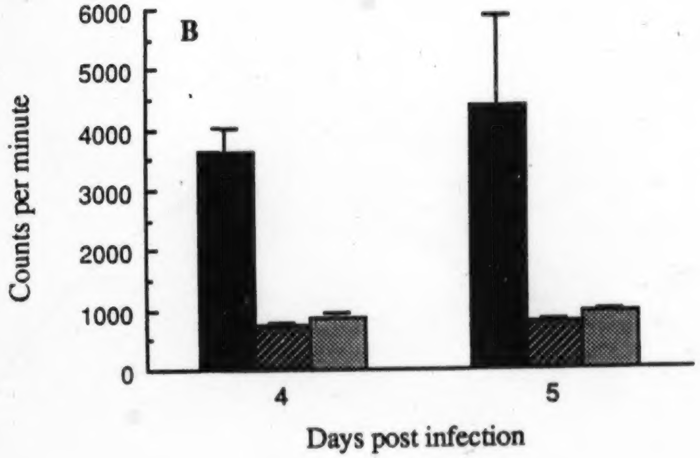
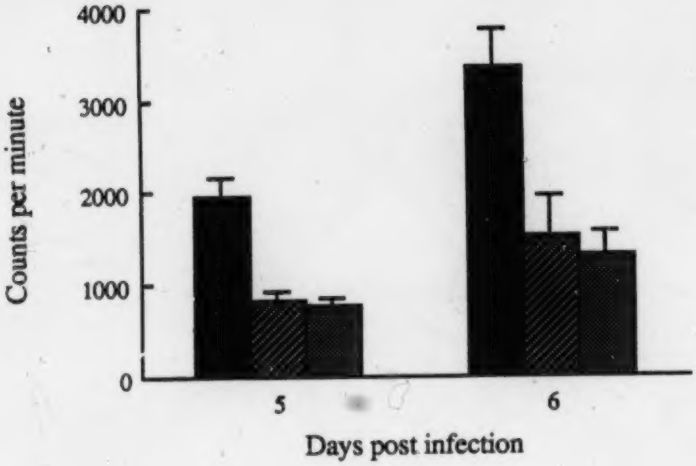

B
Counts per minute
6000
5000
4000
3000
2000
1000
0
4
5
Days post infection

Figure 27. Effect of MV ts38 and MV Lec on the stimulation of PBMC by herpes simplex virus antigens. Cells were infected at a m.o.i. of 5 and stimulated with 10 µg of herpes simplex virus antigen. The cells were incubated for either 5 or 6 days at 37°C and [^{3}H]-thymidine added for the last 18 h of the incubation period. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). Each column represents the mean and standard deviation of six parallel cultures.

● Uninfected PBMC

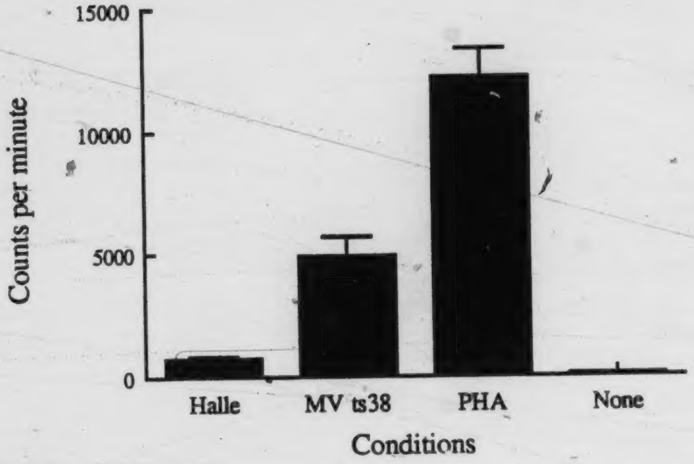
● MV ts38-infected PBMC

▨ MV Lec-infected PBMC


4000
3000
2000
1000
0
Counts per minute
5
6
Days post infection

It is known that thymidine phosphorylase present in PBMC will catabolise thymidine to thymine and deoxyribose-1-phosphate (Perignon *et al.*, 1987). Thus radioactivity from tritiated thymidine would be rendered unincorporable following its catabolism by thymidine phosphorylase. To ensure that the inhibitory effect observed in the presence of MV was real and not due to the breakdown of thymidine, 5-fluorodeoxyuridine (FUdR) and deoxyinosine were used. The presence of FUdR would block the enzyme thymidilate synthetase preventing the methylation of deoxyuridylate to deoxythymidylate (dTMP). The lack of dTMP would be compensated by the radioactive thymidine which would be converted to dTMP by thymidine kinase. Radioactive thymine, a result of radioactive thymidine degradation, could be salvaged in the presence of deoxyinosine by deoxyribosyl transfer (Perignon *et al.* 1987). Thus, in the presence of FUdR and deoxyinosine, DNA synthesis will depend on the presence of radioactive thymidine. Therefore, proliferation assays using two different strains of MV, MV ts38 and MV Halle, were carried out in the presence of FUdR, deoxyinosine and tritiated thymidine, all added for the last 6 h of culture. The results (Figure 28) showed that both MV ts38 and MV Halle blocked thymidine incorporation in the presence of deoxyinosine and FUdR. The percentage inhibition observed for MV ts38 and MV Halle were similar to those observed earlier (Figure 24a and 24b), i.e, MV Halle was found to be more inhibitory than MV ts38.

Figure 28. Effect of MV ts38 and MV Halle on PBMC proliferation in the presence of 5-fluorodeoxyuridine and deoxyinosine. MV ts38 or MV Halle-infected PBMC were incubated at 37°C in the presence of 10 µg/mL of PHA. Eighteen µmol of deoxyinosine and 16 µmol of 5-fluorodeoxyuridine were added together with [^{3}H]-thymidine for the last 6 h of a total incubation time of 72 h. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). Each colum represents the mean and standard deviation of six parallel cultures.


15000
10000
5000
0
Counts per minute
Halle
MV ts38
PHA
None
Conditions

C. Mechanism(s) by which MV ts38 interferes with PBMC responses

1. Role of different subpopulations of PBMC in MV-induced suppression

The relative importance of PBMC subpopulations in bringing about MV-induced inhibitory effect was studied, using enriched subpopulations. The results shown in Figure 29, indicated that while the percentage of inhibition in MV ts38-infected PBMC was 50% by day 4, no significant inhibition was observed in enriched T cell populations infected with MV ts38, and 80% inhibition was observed in enriched monocyte populations. These results implied that monocytes might be of relevance in the process by which the virus brought about the inhibitory effect, while T cells were not involved. The enriched cell populations were obtained by adherence and depletion using monoclonal antibodies and complement against the undesirable cell population, as described in materials and methods.

If monocytes were the main cell types via which the virus exerted the inhibitory effect, a titration effect should be observed when infected monocytes were added in increasing numbers to enriched T cell populations. Different techniques were used to obtain enriched cell populations. Instead of monoclonal antibodies and complement as described above, a percoll gradient was used to obtain enriched

Figure 29. Effect of MV ts38 on the proliferation of PBMC or subsets of PBMC stimulated by PHA. Cells were isolated as described in materials and methods and infected at an m.o.i. of 5. The cells were incubated at 37°C, in the presence of 10 µg/mL of PHA, for different periods of time. [^{3}H]-thymidine was added for the last 18 h of the incubation period. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). Each column represents the mean and standard deviation of six parallel cultures.

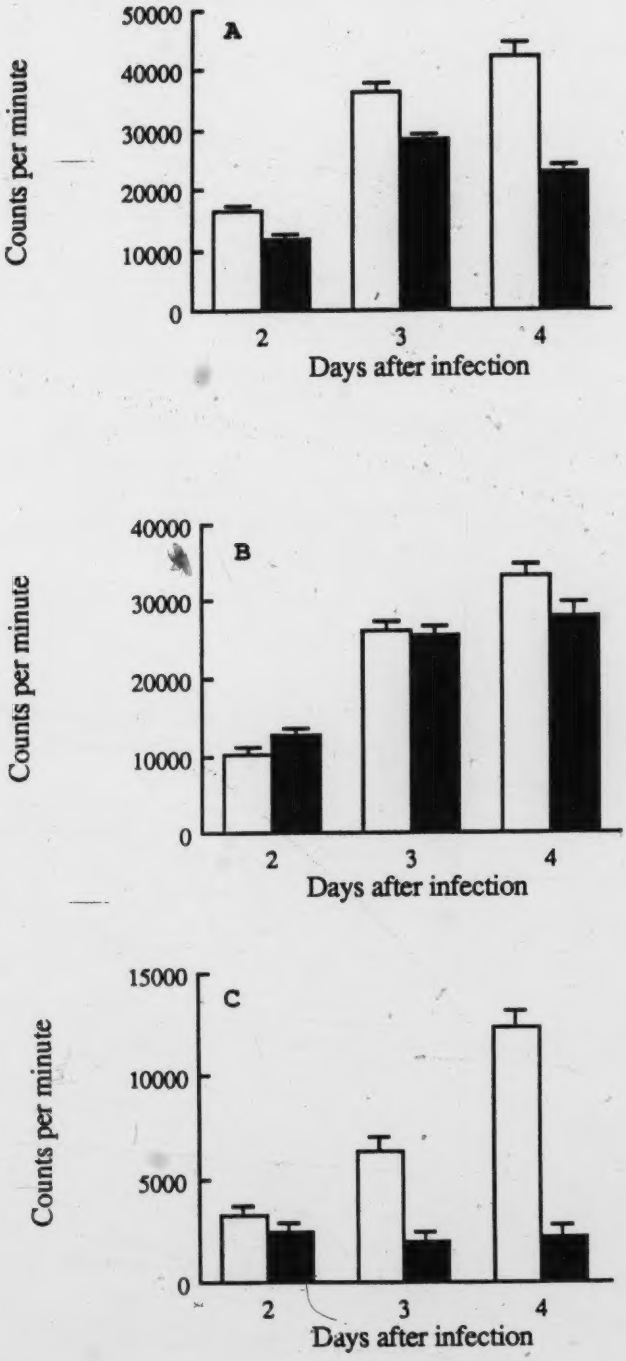
A: Peripheral blood mononuclear cells

B: Enriched T cell populations

C: Enriched monocyte populations

(■) MV ts38-infected cells

(□) Uninfected cells


A
Counts per minute
50000
40000
30000
20000
10000
0
2
3
4
Days after infection
B
Counts per minute
40000
30000
20000
10000
0
2
3
4
Days after infection
C
Counts per minute
15000
10000
5000
0
2
3
4
Days after infection

monocyte populations and for enriched T cell populations, nylon wool columns were used. Figure 30 illustrates the results obtained when uninfected cells from monocyte-enriched populations were added to uninfected cells from enriched T cell populations. Although the proliferation of T cells occured in a cell population depleted of monocytes, indicating the presence of a small number of contaminating monocytes, the addition of cells from monocyte-enriched populations resulted in an increase by 50% in the amount of tritiated thymidine incorporated. When MV ts38-infected cells from monocyte-enriched cell populations were added in increasing numbers, the T cell response decreased accordingly (Figure 31). When infected T cell-enriched or monocyte-enriched cell populations were added to an enriched T cell population that was uninfected, a significant drop in stimulation was observed when the infected, enriched monocyte populations were added and no significant change was observed when infected, enriched T cell populations were added (Figure 32). The results, though consistent with the fact that monocytes were involved in the mechanism(s) by which MV interferes with PBMC proliferation, also indicated that a certain minimum number of monocytes were required for the inhibitory effect to be exerted. From the PHA proliferation experiments (Figure 29), it was clear that a certain number of contaminating monocytes were present in the enriched T cell populations, and these monocytes were not able to bring about any inhibitory effect, i.e, a small number of monocytes

Figure 30. Effect of different number of cells from monocyte-enriched cell population on the proliferation of T cell-enriched populations stimulated by PHA. Different number of uninfected cells from monocyte-enriched cell populations were added to 100,000 cells from T cell-enriched cell populations and incubated at 37°C. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). For each point, mean and standard deviation of six parallel cultures are shown.

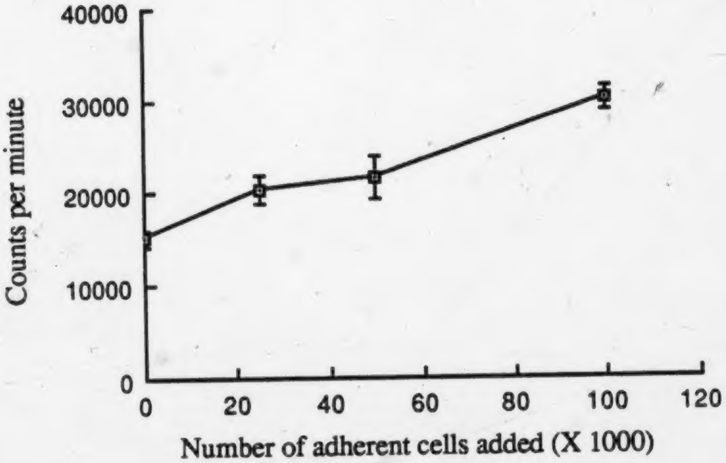

40000
30000
20000
10000
0
Counts per minute
0
20
40
60
80
100
120
Number of adherent cells added (X 1000)

Figure 31. Effect of different number of MV ts38-infected cells from enriched monocyte populations on the proliferation of enriched T cell populations stimulated by PHA. Different number of infected cells (at an m.o.i. of 5) from monocyte-enriched cell populations were added to 100,000 uninfected cells from T cell-enriched cell populations and incubated at 37°C. [^{3}H]-thymidine was added for the last 18 h of a total incubation period of 72 h. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). For each point, the mean and standard deviation of six parallel cultures are shown.

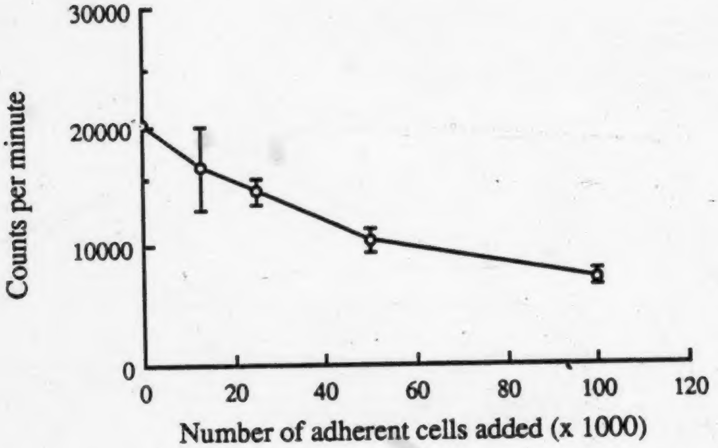
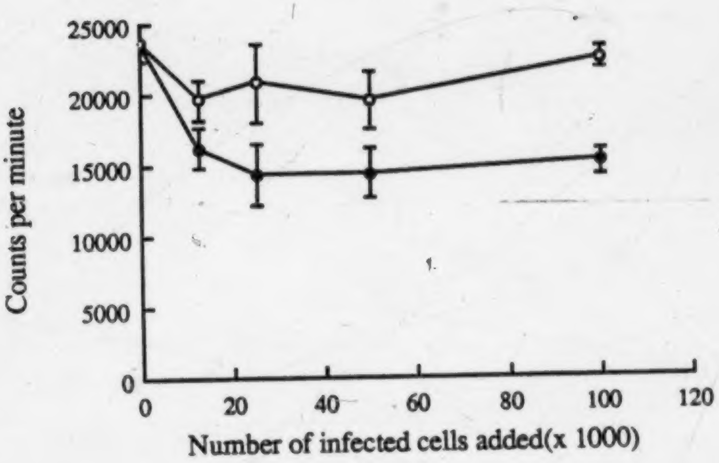

30000
20000
10000
0
Counts per minute
0
20
40
60
80
100
120
Number of adherent cells added (x 1000)

Figure 32. Effect of different number of MV ts38-infected cells from enriched monocyte or enriched T cell populations on the proliferation of uninfected T cell-enriched populations stimulated by PHA. Different number of infected cells from enriched monocyte or enriched T cell populations (both infected at an m.o.i of 5) were added to 100,000 uninfected cells from T cell-enriched populations and incubated at 37°C. [^{3}H]-thymidine was added for the last 18 h of a total incubation time of 72 h. The cells were harvested, the amount of incorporated radioactivity was determined and shown on the Y-axis of the Figure (counts per minute). For each point, the mean and standard deviation of six parallel cultures.

(○)Enriched T cell populations infected with MV ts38

(●)Enriched monocyte populations infected with MV ts38


25000
20000
15000
10000
5000
0
Counts per minute
0
20
40
60
80
100
120
Number of infected cells added(x 1000)

was present, not enough for MV suppression but enough to allow substantial proliferation in the presence of PHA.

One possible explanation for the results described above was that only monocytes were infected by MV ts38. Experiments were set up to determine if both cell types were infected after stimulation. Double immunofluorescence, using specific cell markers and antibodies against MV nucleocapsid, was carried out. The results (Table 3) indicated that both cell types were infected, with higher number of infected T cells than monocytes. From 30% to 40% of infected cells were monocytes and 40 to 50% were found to be T cells. However, the infected T cells and monocytes did not account for all of the infected cells. The remaining infected cells could not be identified with the antisera used but likely consisted of NK and B cells (Jacobson *et al.*, 1984; Casali *et al.*, 1982).

2. Ageing of monocytes

Ageing of monocytes *in vitro* has been shown to increase the efficiency of replication of certain viruses (Narayan *et al.*, 1983; Krilov *et al.*, 1987) and decrease that of others (Linnavuori *et al.*, 1981). To determine if ageing of monocytes might affect the ability of these cells to interfere with T cell proliferation upon infection, enriched monocyte populations were preincubated for different times prior to infection with either MV ts38 or MV Halle. The latter was chosen due to the high inhibitory effect the virus

Table 3. Percentage of T cells and monocytes positive for MV antigens in MV ts38-infected and PHA stimulated PBMC at 37°C.

Expt no	% of infected cells	
	1	2
MMA+ cells	34 ± 5	37 ± 1
OKT3+ cells	44 ± 5	47 ± 2

PBMC were infected with MV ts38 and incubated at 37°C for 48 h. The cells were incubated with either MMA or anti-OKT3 antibodies followed by goat anti-mouse FITC conjugated IgG. The surface labeled cells were sedimented onto glass slides and fixed in acetone at -20°C. The fixed cells were incubated with polyclonal serum against nucleocapsid protein followed by rhodamine labeled goat anti-rabbit IgG. The cells were then examined by fluorescence microscopy. For each experiment, three different regions on each slide were analysed. The number of nucleocapsid-positive cells were counted (100 to 200 cells for each count). The number of MMA or OKT3-positive cells within the nucleocapsid-positive cells were then counted. Each set of data represents the mean and standard deviation determined from three different counts for each experiment.

was able to exert. The enriched monocyte cell populations were then stimulated and the amount of tritiated thymidine incorporated in the presence or absence of virus was monitored. The results are shown in Figure 33, where the percentage inhibition versus the time of incubation prior to infection was plotted. The results demonstrated that preincubation of the monocyte-enriched cell population had no effect on the inhibitory effect of the virus. However, the percentage of inhibition by MV Halle was more than 90% whereas MV ts38 inhibited proliferation by only 45%.

3. Release of infectious virus from enriched T cell and enriched monocyte populations of cells

The importance of monocytes in the inhibitory effect could be due to an increased efficiency of replication in these cells. To study this, the amount of infectious virus released from enriched T cell and enriched monocyte populations at both the permissive and the restrictive temperatures was determined. The results of MV ts38 replication in enriched T cell populations are shown in Figure 34. More than 10^5 infectious particles per mL was detected in PHA-stimulated enriched T cell populations at 32°C, over a period of five days. Both free and cell-associated virus were detected, a similar pattern to that observed in PBMC. At 39°C, no infectious particles were detected after 24 h, indicating that the virus did not undergo a full cycle of replication at that temperature. At

Figure 33. Effect of preincubation of monocyte-enriched cell populations on the inhibitory effect of MV ts38. Monocyte- enriched cell populations were incubated for different times. The cells were infected at an m.o.i. of 5 with MV ts38 and incubated at 37°C, in the presence of 10 μg/mL of PHA for a further 72 h. [^{3}H]-thymidine was added for the last 18 h of the incubation time. The cells were harvested and the amount of incorporated radioactivity determined. The results are expressed as the percentage of inhibition which was calculated as follows: {[Amount of radioactivity incorporated in the absence of virus - Amount of radioactivity incorporated in the presence of virus] ÷ Amount of radioactivity in absence of virus} x 100. Each point represents the mean and standard deviation of six parallel cultures.

(○) MV Halle-infected cells from monocyte-enriched cell populations

(●) MV ts38-infected cells from monocyte-enriched cell populations

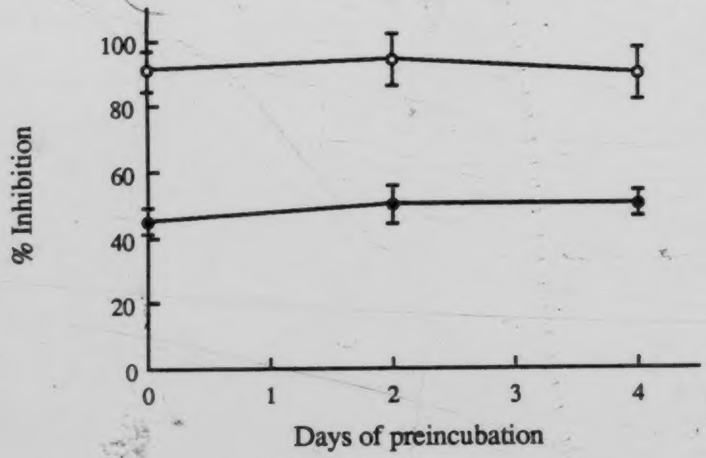

% Inhibition
100
80
60
40
20
0
0
1
2
3
4
Days of preincubation

Figure 34. Amount of infectious virus detected in supernatant solutions and cells of MV ts38-infected T cell-enriched populations grown at 32°C, 37°C and 39°C in the presence or absence of PHA. Cells and supernatant solutions were harvested at different times and tested for the presence of infectious virus by plaque assay at 32°C and 39°C. The results shown are from the plaque assays at 32°C. No plaques were obtained at 39°C. The amounts of infectious virus are shown on a logarithmic scale.

A: Amount of infectious virus in supernatant solutions

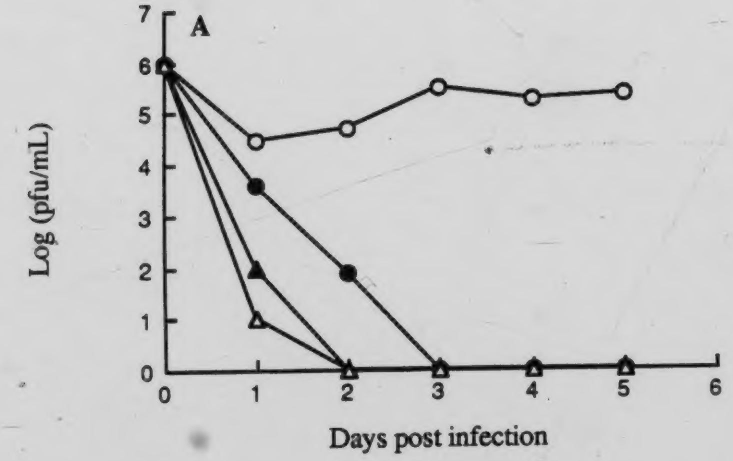
B: Amount of cell-associated virus

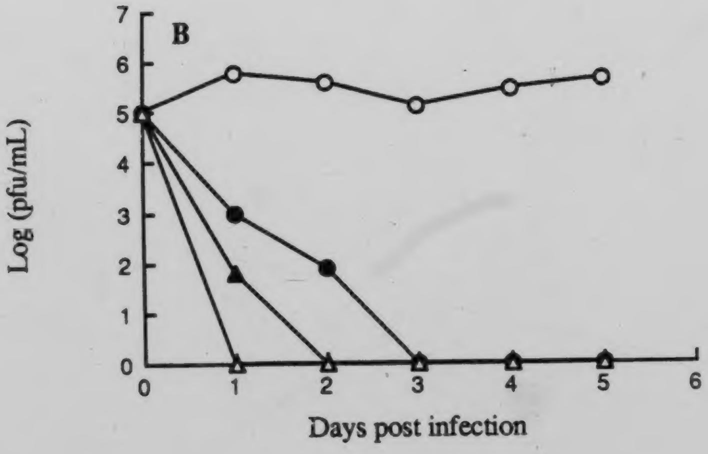
(○) MV ts38-infected and PHA-stimulated cells from T cell-enriched populations. The cells were incubated at 32°C

(●) MV ts38-infected and PHA-stimulated cells from T cell-enriched populations. The cells were incubated at 37°C

(▲) MV ts38-infected and PHA-stimulated cells from T cell-enriched populations. The cells were incubated at 39°C

(△) Unstimulated, MV ts38-infected cells from T cell-enriched populations. The cells were incubated at 37°C


A
Log (pfu/mL)
0
1
2
3
4
5
6
7
Days post infection
0
1
2
3
4
5
6


B
Log (pfu/mL)
0
1
2
3
4
5
6
7
Days post infection
0
1
2
3
4
5
6

37°C, on the other hand, residual infectious particles could be detected by day 2. The amount of infectious particles detected, showed a constant decrease over time, with no infectivity by day 3. The fact that a constant decrease over time was observed meant that the infectious particles detected were part of the original inoculum, though the possibility that these particles were newly synthesized could not be disproved.

In LPS-stimulated enriched monocyte populations, the amount of infectious particles detected at any one time at the permissive temperature was lower than at similar times in enriched T cell populations (Figure 35). In fact, the amount of infectious virus detected in these cells never did reach the level observed for enriched T cell populations. Replication occurred only at 32°C, even then at a reduced level compared to enriched T cell populations. At 37°C and 39°C, infectious particles could be detected only within the first 24 h. Similar results were obtained for unstimulated enriched T cell and enriched monocyte populations, i.e, infectious particles could be detected only within 24 h. These results indicated that the efficiency of MV ts38 replication at the permissive temperature was better in enriched T cell populations than in enriched monocyte populations and that in both cell types, the temperature sensitivity was retained at 37°C and 39°C.

Figure 35. Amount of infectious virus detected in supernatant solutions and cells of MV ts38-infected monocyte-enriched populations, grown at 32°C, 37°C and 39°C in the presence or absence of LPS. Cells and supernatant solutions were harvested at different times and tested for the presence of infectious virus by plaque assay at 32°C and 39°C. The results shown are from the plaque assays at 32°C. No plaques were obtained at 39°C. The amounts of infectious virus are shown on a logarithmic scale.

A: Amount of infectious virus in supernatant solutions

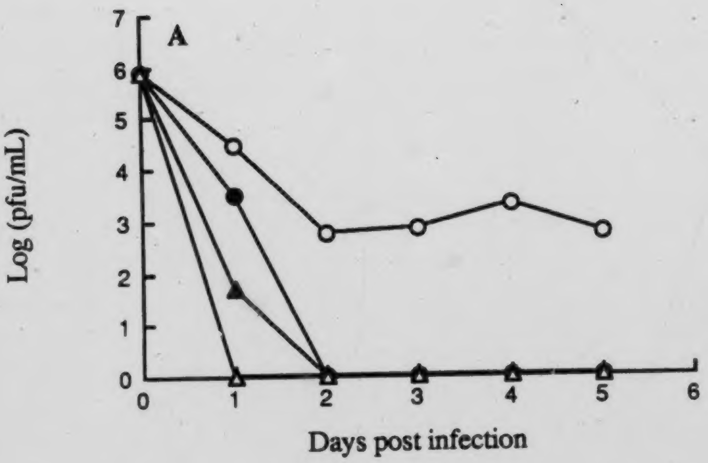
B: Amount of cell-associated virus

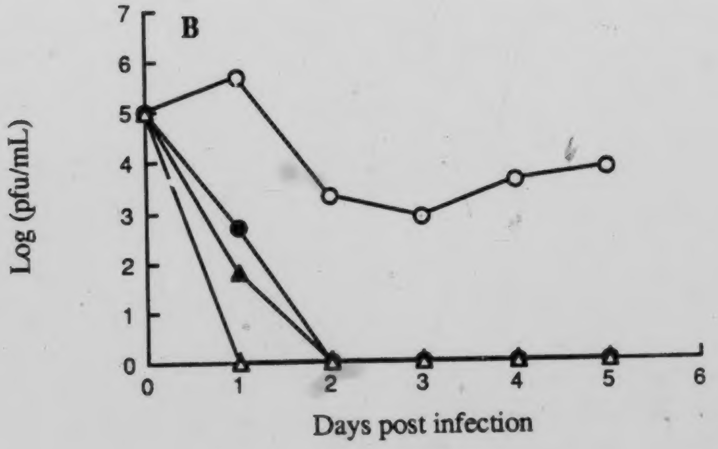
(○) MV ts38-infected and LPS-stimulated cells from monocyte-enriched populations. The cells were incubated at 32°C

(●) MV ts38-infected and LPS-stimulated cells from monocyte-enriched populations. The cells were incubated at 37°C

(▲) MV ts38-infected and LPS-stimulated cells from monocyte-enriched populations. The cells were incubated at 39°C

(△) Unstimulated, MV ts38-infected cells from monocyte-enriched populations. The cells were incubated at 37°C


A
Log (pfu/mL)
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Days post infection


B
Log (pfu/mL)
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Days post infection

In comparison, in enriched T cell populations infected with MV Lec, infectious particles could be detected for up to five days at 32°C though in much lower numbers than in enriched T cell populations infected with MV ts38, especially by day 5 (Figure 36). At 37°C, infectious particles were also observed up to day 5 in MV Lec-infected enriched T cell populations, in contrast to MV ts38-infected enriched T cell populations. At 39°C, infectious particles could be detected for up to 3 days only.

This was still different than MV ts38-infected, enriched T cell population where infectivity was reduced to zero within 24 h. The replication of MV Lec in enriched monocyte populations was not as efficient as in enriched T cell populations (Figure 37). Infectious particles could be detected in MV Lec-infected enriched monocyte populations up to day 5 at 32°C, in comparative amounts to MV ts38-infected enriched monocyte populations at the same temperature, but significantly lower than in infected, enriched T cell populations. At 37°C, infectious particles were detected for up to four days, and at 39°C, up to 72 h. These results clearly indicated that the efficiency of replication of MV ts38 and MV Lec in activated, enriched T cell populations was significantly higher than in activated enriched monocyte populations.

Figure 36. Amount of infectious virus detected in supernatant solutions and cells of MV Lec-infected T cell-enriched populations grown at 32°C, 37°C and 39°C in the presence or absence of PHA. Cells and supernatant solutions were harvested at different times and tested for the presence of infectious virus by plaque assay at 32°C and 39°C. The results shown are from the plaque assays at 32°C. No plaques were obtained at 39°C. The amounts of infectious virus detected are shown on a logarithmic scale.

A: Amount of infectious virus in supernatant solutions

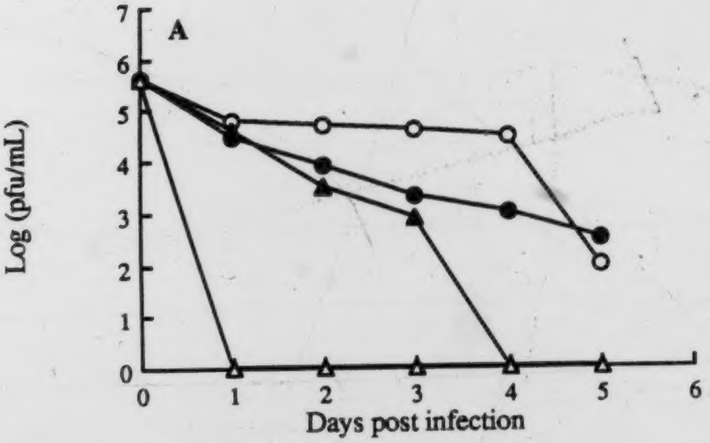
B: Amount of cell-associated virus

(○) MV Lec-infected and PHA-stimulated cells from T cell-enriched populations. The cells were incubated at 32°C

(●) MV Lec-infected and PHA-stimulated cells from T cell-enriched populations. The cells were incubated at 37°C

(▲) MV Lec-infected and PHA-stimulated cells from T cell-enriched populations. The cells were incubated at 39°C

(△) Unstimulated, MV Lec-infected cells from T cell-enriched populations. The cells were incubated at 37°C


A
Log (pfu/mL)
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Days post infection

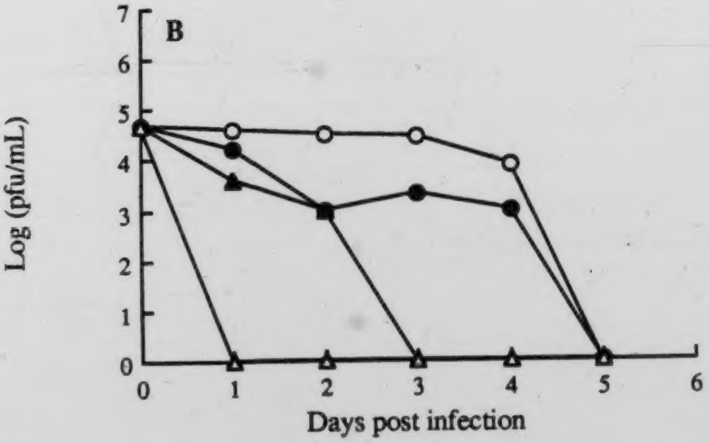

B
Log (pfu/mL)
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Days post infection

Figure 37. Amount of infectious virus detected in supernatant solutions and cells of MV Lec-infected monocyte-enriched populations, grown at 32°C, 37°C and 39°C in the presence or absence of LPS. Cells and supernatant solutions were harvested at different times and tested for the presence of infectious virus by plaque assay at 32°C and 39°C. The results shown are from the plaque assays at 32°C. No plaques were obtained at 39°C. The amounts of infectious virus are shown on a logarithmic scale.

A: Amount of infectious virus in supernatant solutions

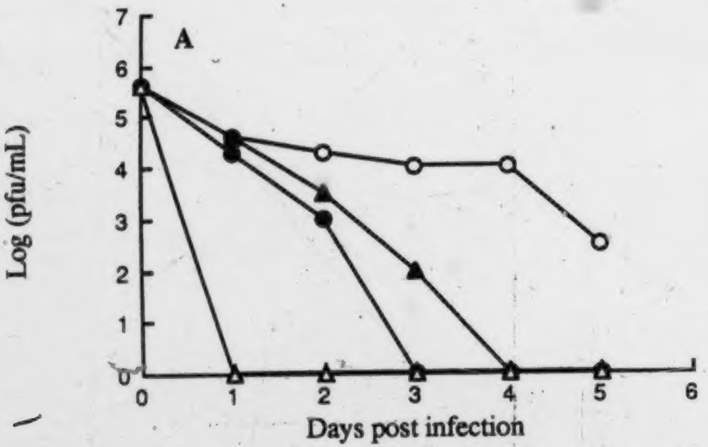
B: Amount of cell-associated virus

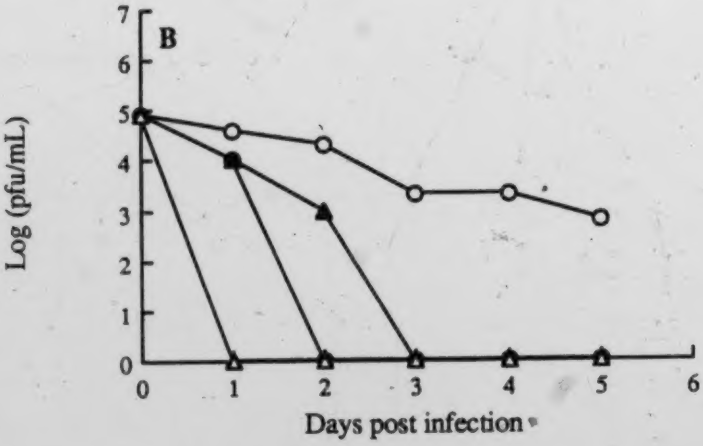
(○) MV Lec-infected and LPS-stimulated cells from monocyte-enriched populations. The cells were incubated at 32°C

(●) MV Lec-infected and LPS-stimulated cells from monocyte-enriched populations. The cells were incubated at 37°C

(▲) MV Lec-infected and LPS-stimulated cells from monocyte-enriched populations. The cells were incubated at 39°C

(△) Unstimulated, MV Lec-infected cells from monocyte-enriched populations. The cells were incubated at 37°C


A
Log (pfu/mL)
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Days post infection


B
Log (pfu/mL)
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Days post infection

4. Viral mRNA in enriched T cell and enriched monocyte populations of cells

Experiments were carried out to determine if more efficient transcription took place in enriched T cell populations than in enriched monocyte populations. RNA was isolated from both MV ts38-infected enriched T cell and enriched monocyte cell populations and hybridizations, using slot blots, were carried out. Probes for the N, M and H (+) strand RNA were used and the results, as shown in Figures 38 to 41, indicated that at both 32°C and 37°C, transcription was taking place in both populations of cells infected with MV ts38. This was similar to the results obtained in PBMC (Figures 11 to 13). Viral messages could be detected when a minimum of 80 ng of total RNA was used. No significant difference in the level of transcription was observed between the two cell populations, when probes detecting N (Figure 38), M (Figure 39) or H (Figure 40) positive strand RNA were used. A probe of positive polarity for the M gene was used to determine if genomic RNA was present. The results (Figure 41) showed that genomic RNA was present in both enriched T cell and enriched monocyte populations in similar amounts.

Figure 38. Detection of MV (+) strand RNA of the N gene by slot blot analysis of total RNA isolated from MV ts38-infected T cell-enriched and monocyte-enriched populations of cells. Total RNA, isolated by a rapid method, was denatured and spotted onto nylon membrane by the use of a slot blot apparatus as described in materials and methods. Ten μg of denatured RNA in a total volume of 100 μL was spotted for wells #1. Two-fold serial dilutions were carried out in 10 x SSC and used for wells #2 to 8. Prehybridization and hybridization were carried out, as described in materials and methods, using a single stranded MV N gene of (-) polarity at a concentration of 10^6 to 10^7 cpm/mL. The blot was then washed and autoradiographed.

Lane A: Total RNA isolated from Edmonston-infected Vero cells

Lane B: Total RNA isolated from uninfected Vero cells

Lane C: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 32°C

Lane D: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 37°C

Lane E: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 32°C.

Lane F: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 37°C

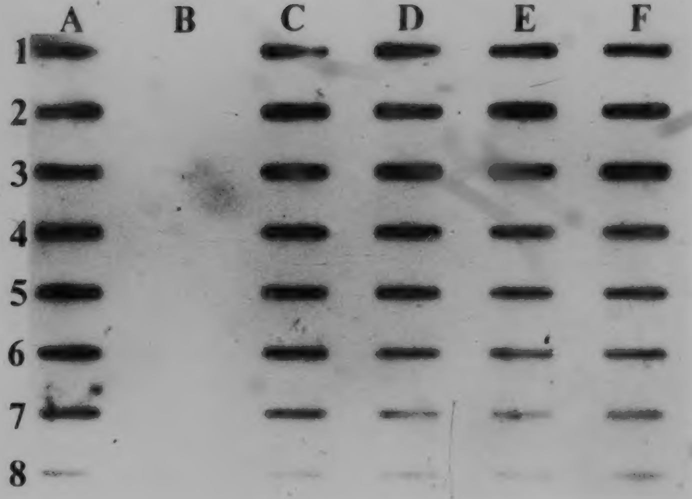

A
B
C
D
E
F
1
2
3
4
5
6
7
8

Figure 39. Detection of MV (+) strand RNA of the M gene by slot blot analysis of total RNA isolated from MV ts38-infected T cell-enriched and monocyte-enriched populations of cells. Total RNA, isolated by a rapid method, was denatured and spotted onto nylon membrane by the use of a slot blot apparatus as described in materials and methods. Ten µg of denatured RNA in a total volume of 100 µL was spotted for wells #1. Two-fold serial dilutions were carried out in 10 x SSC and used for wells #2 to 8. Prehybridization and hybridization were carried out, as described in materials and methods, using a single stranded MV M gene of (-) polarity at a concentration of 10^6 to 10^7 cpm/mL. The blot was then washed and autoradiographed.

Lane A: Total RNA isolated from Edmonston-infected Vero cells

Lane B: Total RNA isolated from uninfected Vero cells

Lane C: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 32°C

Lane D: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 37°C

Lane E: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 32°C.

Lane F: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 37°C

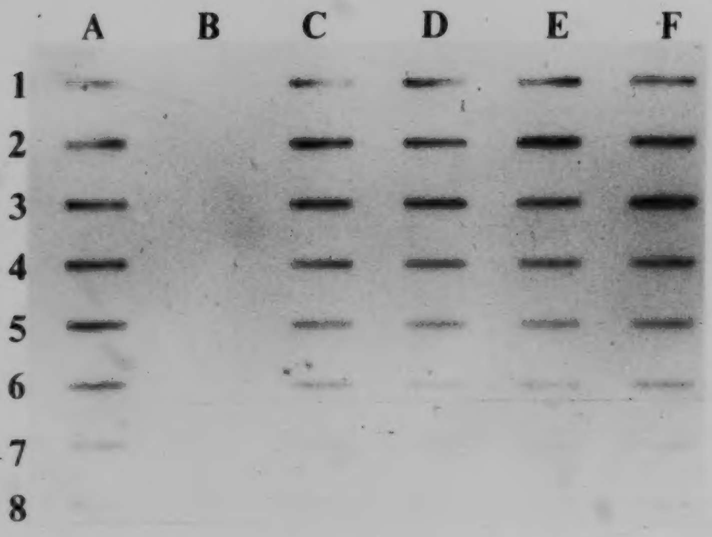

A
B
C
D
E
F
1
2
3
4
5
6
7
8

Figure 40. Detection of MV (+) strand RNA of the H gene by slot blot analysis of total RNA isolated from MV ts38-infected T cell-enriched and monocyte-enriched populations of cells. Total RNA, isolated by a rapid method, was denatured and spotted onto nylon membrane by the use of a slot blot apparatus as described in materials and methods. Ten µg of denatured RNA in a total volume of 100 µL was spotted for wells #1. Two-fold serial dilutions were carried out in 10 x SSC and used for wells # 2 to 8. Prehybridization and hybridization were carried out as described in materials and methods using a single stranded MV H gene of (-) polarity at a concentration of 10^6 to 10^7 cpm/mL. The blot was then washed and autoradiographed.

Lane A: Total RNA isolated from Edmonston-infected Vero cells

Lane B: Total RNA isolated from uninfected Vero cells

Lane C: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 32°C

Lane D: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 37°C

Lane E: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 32°C.

Lane F: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 37°C

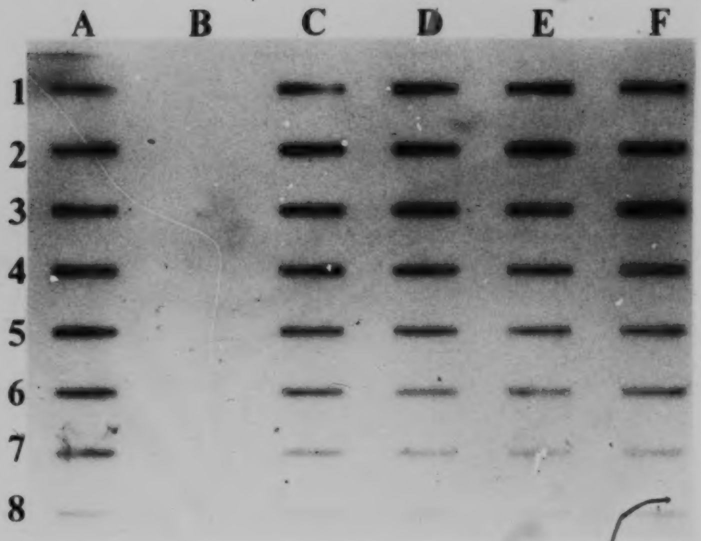

A
B
C
D
E
F
1
2
3
4
5
6
7
8

Figure 41. Detection of MV genomic RNA by slot blot analysis of total RNA isolated from MV ts38-infected T cell-enriched and monocyte-enriched populations of cells. Total RNA, isolated by a rapid method, was denatured and spotted onto nylon membrane by the use of a slot blot apparatus as described in materials and methods. Ten µg of denatured RNA in a total volume of 100 µL was spotted for wells #1. Two-fold serial dilutions were carried out in 10 x SSC and used for wells # 2 to 8. Prehybridization and hybridization were carried out as described in materials and methods using a single stranded MV M gene of (+) polarity at a concentration of 10^6 to 10^7 cpm/mL. The blot aws then washed and autoradiographed.

Lane A: Total RNA isolated from Edmonston-infected Vero cells

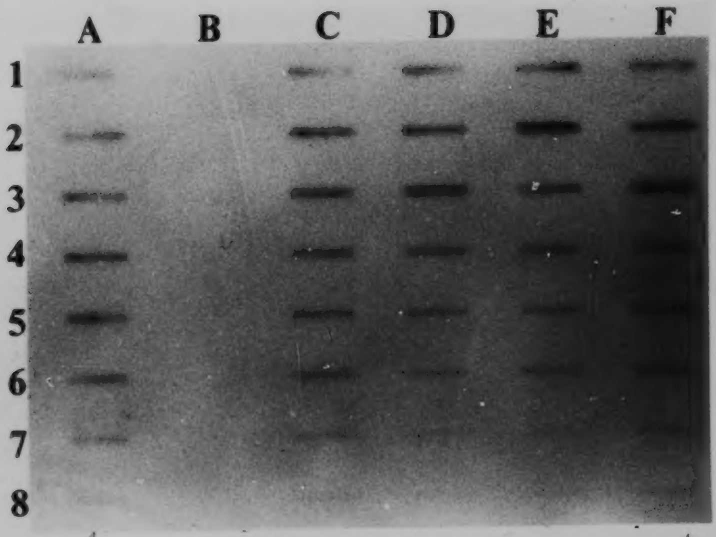
Lane B: Total RNA isolated from uninfected Vero cells

Lane C: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 32°C

Lane D: Total RNA isolated from enriched T cell populations infected with MV ts38 and grown at 37°C

Lane E: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 32°C.

Lane F: Total RNA isolated from enriched monocyte populations infected with MV ts38 and grown at 37°C


A
B
C
D
E
F
1
2
3
4
5
6
7
8

5. Viral protein synthesis in enriched T cell and enriched monocyte populations of cells.

In both enriched T cell and enriched monocyte populations, MV ts38 replication was restricted at the nonpermissive temperatures. To determine if viral proteins were being synthesized in both cell populations, radioimmunoprecipitation followed by gel electrophoresis was carried out. The results showed that, for all temperatures tested, full size viral proteins were being synthesized in enriched T cell populations (Figure 42). Six bands, H, P, N, A, F_1 and M could be seen. No difference was observed when the protein profiles of enriched T cell populations, infected with MV ts38 and incubated at different temperatures, were compared. The viral proteins synthesized in enriched monocyte populations are shown in Figure 43. Six viral proteins were detected at all three temperatures. The viral proteins detected were H, P, N, A, F_1 and M. Moreover, no significant difference in viral protein profiles was observed at the different temperatures tested. In both enriched T cell and enriched monocyte populations, full size viral proteins were being synthesized. The amount of viral proteins synthesized at 32°C, 37°C and 39°C in enriched T cell and enriched monocyte populations were measured by enzyme immunoassays, as described in materials and methods. Two viral proteins were measured and the results (Table 4) indicated that

Figure 42. Presence of MV proteins at 32°C, 37°C and 39°C in MV ts38-infected T cell-enriched populations. Cells were infected at an m.o.i. of 5 and grown at 32°C, 37°C or 39°C in medium containing 37,000 KBq/mL of [^{35}S]-methionine and 10 µg/mL of PHA, as described in materials and methods. The cells were harvested 32 h later. Viral proteins were immunoprecipitated with anti-MV polyclonal serum and electrophoresed in an SDS-polyacrylamide gel. The gel was then dried under vacuum and autoradiographed as described in materials and methods.

A: Viral proteins from MV ts38-infected T cell-enriched populations incubated at 32°C

B: Viral proteins from MV ts38-infected T cell-enriched populations incubated at 37°C

C: Viral proteins from MV ts38-infected T cell-enriched populations incubated at 39°C.

D: Viral proteins from uninfected T cell-enriched populations incubated at 37°C

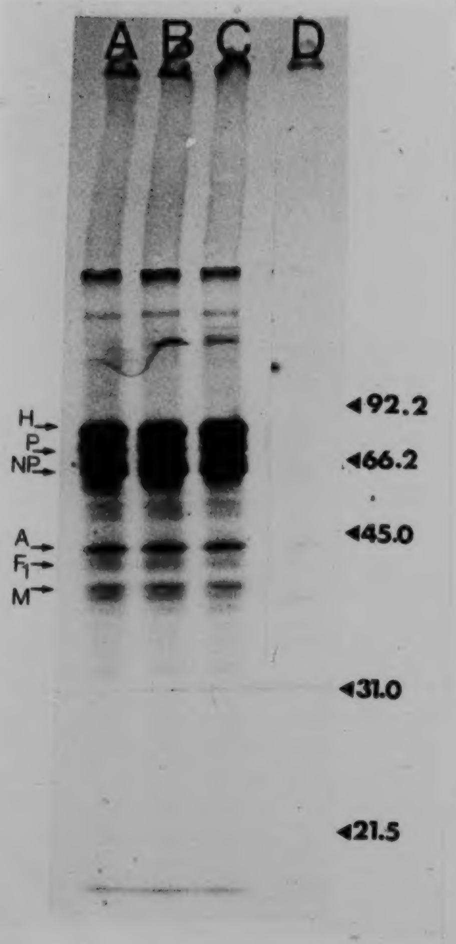

A
B
C
D
H
P
NP
A
F1
M
92.2
66.2
45.0
31.0
21.5

Figure 43. Presence of MV proteins at 32°C, 37°C and 39°C in MV ts38-infected monocyte-enriched populations. Cells were infected at an m.o.i. of 3 to 5 and grown at 32°C, 37°C or 39°C in medium containing 37,000 KBq/mL of [^{35}S]-methionine and 25 µg/mL of LPS, as described in materials and methods. The cells were harvested 32 to 34 h later. Viral proteins were immunoprecipitated with anti-MV polyclonal serum and electrophoresed in an SDS-polyacrylamide gel. The gel was then dried under vacuum and autoradiographed.

A: Viral proteins from uninfected monocyte-enriched populations incubated at 37°C

B: Blank lane

C: Viral proteins from MV ts38-infected monocyte-enriched populations incubated at 32°C.

D: Viral proteins from MV ts38-infected T cell-enriched populations incubated at 37°C

E: Viral proteins from MV ts38-infected monocyte-enriched populations incubated at 39°C

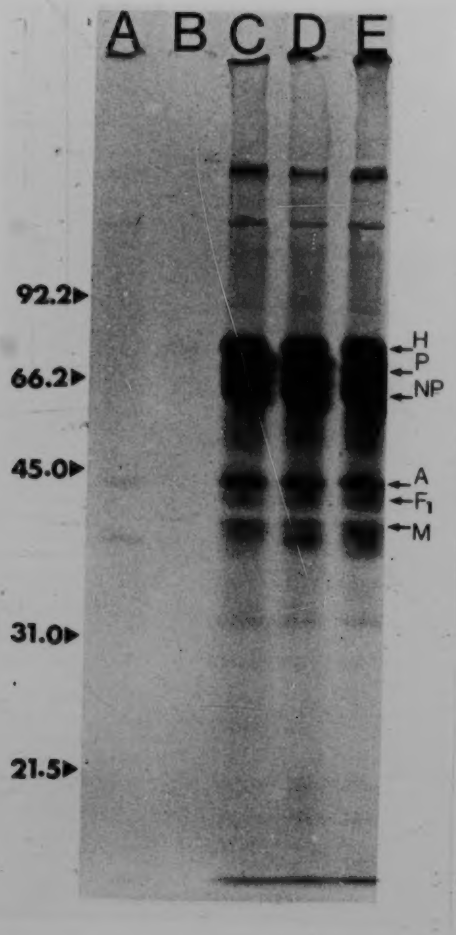

A
B
C
D
E
92.2
66.2
45.0
31.0
21.5
H
P
NP
A
F_1
M

Table 4. Amount of different viral proteins present at 24 h post infection in T cell-enriched and monocyte-enriched populations infected with MV ts38 and grown at different temperatures.

Viral	T cells			Monocytes		
Protein	32°C	37°C	39°C	32°C	37°C	39°C
N	18±2*	25±4	26±3.5	19±2	18±2	15±3.5
H	12.2±1.3	11.2±1.3	11.2±2	10.2±2.0	8.9±1.0	12±1.3

* expressed as percentage of cell lysate antigen.

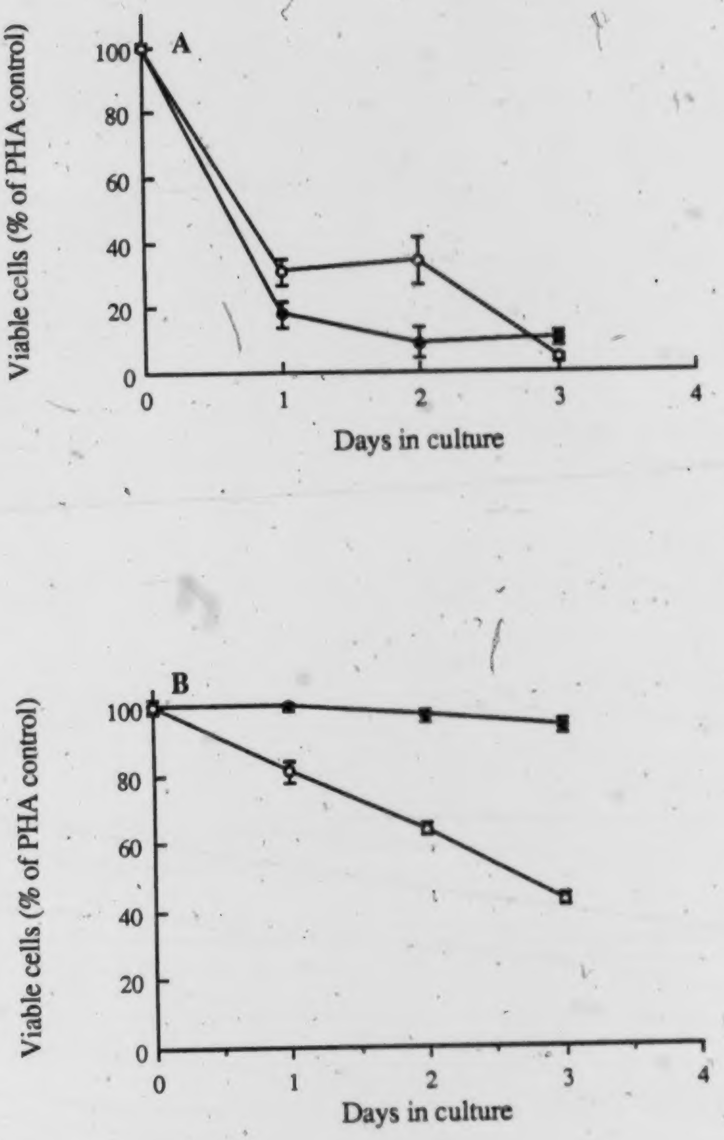
EIA were performed as described in materials and methods. Cell lysate antigen was used to standardize the system. A standard curve was drawn from OD results obtained when different dilutions of cell lysate antigen were used. MV ts38-infected T cell-enriched and monocyte-enriched populations were tested in a 1:2 dilution. The OD results obtained from the samples were compared to the standard curve. The results are expressed as the percentage of MV protein present in cell lysate antigen at a dilution of 1:50. Means and standard deviations were calculated from triplicate plates.

significantly more N than H was synthesized in both populations of cells. No significant difference was observed in the amount of the two viral proteins in enriched T cell populations when compared to enriched monocyte populations. Similarly, no significant difference was observed in the amount of viral protein synthesized at the three different temperatures tested.

6. Effect of MV infection on cell survival

It has been proposed that the cytopathic effect of the virus resulting in cell death might be responsible for the immunosuppressive effect or at least contribute to it (Borysiewick *et al.*, 1985; Salonen *et al.*, 1989). However, no experimental evidence has ever been provided for or against the hypothesis. Using MV ts38, the importance of cell death as a contributing factor to the inhibitory effect was assessed. The percentage of cell death at both the permissive and the restrictive temperatures was determined. Viable cell counts, using the vital dye, Trypan Blue, were obtained and the results are illustrated in Figure 44. Figure 44A shows the percentage of viable cells at 32°C in the presence of MV ts38 or MV Lec. Both viruses significantly reduced the percentage of viable cells to within 10% of the control. However, at 37°C (Figure 44B), MV Lec reduced cell viability to 40% of the control, while at the same temperature, MV ts38 did not significantly reduce the percentage of live cells.

Figure 44. Effect of MV infection on the viability of PBMC. Cells were infected at a m.o.i. of 5 with either MV ts38 (●) or MV Lec (○). The cells were stimulated with PHA and incubated at either (A) 32°C or (B) 37°C. At different times post incubation, cells were harvested and counted by Trypan Blue exclusion. The results are expressed as percentage of viable cells in PHA-stimulated, uninfected cultures. Each point represents the mean and standard deviation of four parallel cultures.


A
Viable cells (% of PHA control)
100
80
60
40
20
0
0
1
2
3
4
Days in culture
B
Viable cells (% of PHA control)
100
80
60
40
20
0
0
1
2
3
4
Days in culture

For both the mutant and the wild type, there was more cell death at 32°C than at 37°C. While, both MV Lec and MV ts38 could inhibit PBMC proliferation at 37°C, MV ts38 did not significantly reduce the number of viable cells, whereas MV Lec reduced it by 60%. These results led to the conclusions that MV ts38 abrogate PBMC proliferation without killing the cells. However, cell death is a contributing factor in MV Lec-induced inhibitory effect.

7. Level of IL-2 in MV ts38-infected PBMC

IL-2 is an immunological mediator that has been shown to be affected during some viral infections, e.g, Sendai virus and RSV (Mims *et al.*, 1986). In order to assess the role of IL-2 in MV inhibition of PBMC proliferation, the level of IL-2 in uninfected and MV ts38-infected PBMC was compared, using an IL-2 dependent cell line. As shown in Figure 45, no difference was observed in the level of IL-2 between uninfected and infected PBMC. These results indicated that the secretion of IL-2 was not affected by MV ts38 infection.

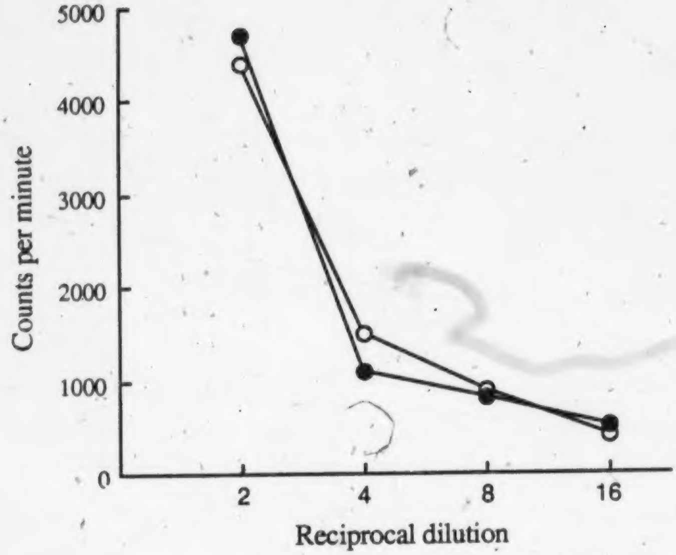
8. Release of IFNα and its role in MV ts38 inhibitory effect

The release of IFNα upon interaction of viruses with PBMC is a well known phenomenon. The amount of IFNα in MV ts38-infected PBMC (10^6/mL) at 37°C was found to be within the range of 100-500 IU/mL. To determine if IFNα was one of

Figure 45. Amount of IL-2 detected in MV ts38-infected and uninfected, PHA-stimulated PBMC. Cell were infected with MV ts38 at an m.o.i. of 3 to 5, stimulated with 10 µg/mL of PHA and incubated at 37°C. The supernatant solutions of the cultures were harvested at 24 h p.i. and added in different dilutions to wells containing 20,000 IL-2-dependent cells. [^{3}H]-thymidine was added 6 h later and incubated for another 18 h. Cell were then harvested and the amount of incorporated radioactivity determined. The results are expressed as the amount of radioactivity incorporated. Each point represents the mean of six parallel cultures.

(●) PHA-stimulated, uninfected

(○) PHA-stimulated, MV ts38 infected


5000
4000
3000
2000
1000
0
Counts per minute
2
4
8
16
Reciprocal dilution

the mediators by which MV ts38 achieved its inhibitory effect, anti-IFNα was used. A titration was performed to determine the amount of anti- IFNα required for the complete neutralization of IFNα present in MV ts38-infected and PHA stimulated PBMC. Four thousand five hundred NU/mL was found to neutralise all of the IFNα present in these samples. As shown in Table 5, 4500 NU/mL of anti-IFNα reversed the inhibitory effect substantially, though not completely. These results suggested that IFNα is one of the mediators via which MV interfered with PBMC proliferation.

9. Effect of MV proteins on PBMC stimulation by PHA

It has been reported that certain viral proteins could be immunosuppressive (Synderman *et al.*, 1985). A well known example is the P15 protein of the envelope of feline leukemia virus (Mathes *et al.*, 1979). To determine if MV proteins have an inhibitory effect on PBMC proliferation or whether an infection is required, inactivated virus was used. Inactivated MV ts38 was added to PHA stimulated PBMC cultures in vitro and tritiated thymidine incorporation was monitored at days 2, 3 and 4. The results shown in Table 6, indicated that no significant difference was observed in presence or absence of inactivated MV ts38 over the four-day period. These results meant that MV proteins were not involved in the inhibitory effect under the test conditions used and that an infection was required for suppression to occur.

Table 5. Effect of anti IFN-α on the inhibitory effect of MV ts38 mutant.

	NO ANTIBODY		ANTI-IFN-α	
	UNINFECTED	MVts38 INFECTED	UNINFECTED	MVts38 INFECTED
EXPERIMENT 1				
DAY 2	14240±582	11512±624	15021±312	13126±756
DAY 3	20562±865	13212±842	20968±517	18612±935
EXPERIMENT 2				
DAY 2	13531±985	9962±837	13901±562	11852±780
DAY 3	21542±1023	14760±472	21908±614	18520±921

MV ts38-infected (m.o.i. of 3 to 5) or uninfected PBMC were added to 96-well plates at a concentration of 10^6 cells/mL in the presence of 10 µg/mL of PHA. Anti interferon-α at a concentration of 4500 NU/mL was added. The same dilution of control serum (as that used to obtain 4500 NU/mL of anti interferon-α) was added to the control cultures. [^{3}H]-thymidine was added to the cultures for the last 18 h of a total incubation period of either 2 or 3 days at 37°C. The cells were then harvested and the amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. Mean and standard deviation of six parallel cultures are shown. Data shown are the results of two representative experiments.

Table 6. Effect of inactivated MV ts38 on the proliferation of PBMC in vitro.

Condition	DAY 2	DAY 3	DAY 4
PHA + Virus	20094 ± 2108	26986 ± 2117	35096 ± 2082
PHA	21258 ± 2407	28509 ± 17[illegible]	34967 ± 2604
Control	324 ± 39	418 ± [illegible]	403 ± 82

MV ts38 was inactivated by UV-irradiation and added to PBMC that were then stimulated with PHA at 10 µg/mL. For every cell, 5 infectious particles were inactivated and used. The cells were incubated at 37°C for 2, 3 or 4 days. [^{3}H]-thymidine was added for the last 18 h of the incubation period. The cells were harvested and the amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. Mean and standard deviation of six parallel cultures are shown. Prior to use, the inactivated virus was tested by plaque assay to ensure lack of infectivity within the inactivated virus preparation.

D. Presence of DNA-synthesis inhibitory molecules in supernatant solutions from MV-infected PBMC

1. Effect of supernatant solutions from MV-infected PBMC on continuous cell lines

Preliminary experiments were performed to develop and determine the best conditions for an assay system to study the inhibitory factors present in the SN of MV-infected PBMC. The well size, number of cells per well, time of addition of the SN and of tritiated thymidine as well as the total incubation time were the parameters that were established for optimal, reproducible test conditions. The test system described below gave consistent DNA-suppression with MV-infected PBMC SN.

SN to be tested were added to 96-well plates seeded 24 h earlier with 25,000 cells per well. [^{3}H]-thymidine was then added for the last 18 h for a total incubation time of 48 h. These conditions have been chosen based on tests performed using three different cell lines, Vero, HeLa, and HEp-2 cells, and have been used for all the tests described.

Earlier results indicated that the extent by which PBMC proliferation was blocked by MV varied, depending upon the strain used. To study this question further, four different strains, namely, Edmonston, Halle, Lec, and MV ts38 were used. SN obtained from infected PBMC were tested on Vero, HeLa and HEp-2 cell lines. Of these, HeLa cells were the most affected (Figures 46 to 48), though both of the other cell lines were also affected, albeit to a much lesser extent. SN harvested 48 h p.i., were more potent in their inhibition of cell growth, though SN harvested after 24 and 72 h were also inhibitory. SN obtained from PBMC infected with different MV strains were not significantly different in their inhibitory effect on HeLa cells.

The effect of the different SN are shown in Figure 49, where different dilutions of the SN were tested on HeLa cells. An inhibitory effect could be observed and the effect decreased with increasing dilution. However, no significant difference was observed between the different strains used, confirming the earlier results obtained with the cell assay.

2. Effect of temperature on the generation of DNA-synthesis inhibitory molecules

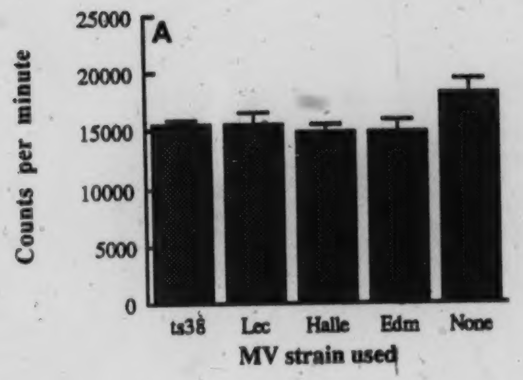
Our previous results indicated that large amounts of infectious virus could be detected at 32°C, in MV ts38-infected PBMC, whereas the virus did not undergo a full cycle of virus replication at 37°C. Thus, experiments were designed to determine if a full cycle of virus replication was required for SN from MV ts38-infected PBMC, to block DNA

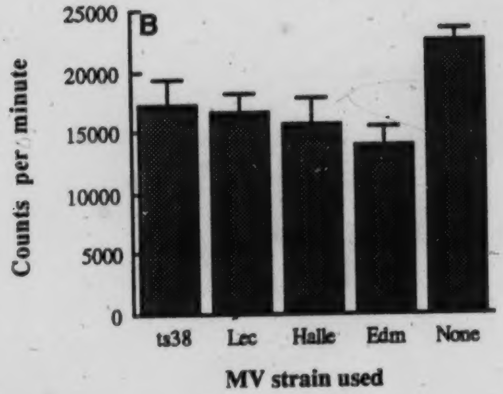
Figure 46. Effect of supernatant solutions (SN) harvested at different times on the growth of Vero cells in vitro. SN were harvested from PHA-stimulated PBMC infected with different strains of MV (MV ts38: ts38; MV Lec: Lec; MV Halle: Halle; MV Edmonston: Edm; uninfected cells: None). SN were then UV-irradiated and ultracentrifuged as described in materials and methods. Tests for infectivity of these SN were negative. One hundred µL of SN were added to confluent monolayers of Vero cells, grown for 24 h in 96-well plates (25,000 cells/well) as described in materials and methods. [^{3}H]-thymidine was added for the last 6 h of a total incubation period of 48 h. The cells were then lifted off the surface of the plates by a trypsin solution and harvested as described in materials and methods. The amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. Each column represents the mean and standard deviation of six parallel cultures.

A: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 24 h of incubation.

B: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 48 h of incubation.

C: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 72 h of incubation.


A
Counts per minute
25000
20000
15000
10000
5000
0
ts38
Lec
Halle
Edm
None
MV strain used


B
Counts per minute
25000
20000
15000
10000
5000
0
ts38
Lec
Halle
Edm
None
MV strain used

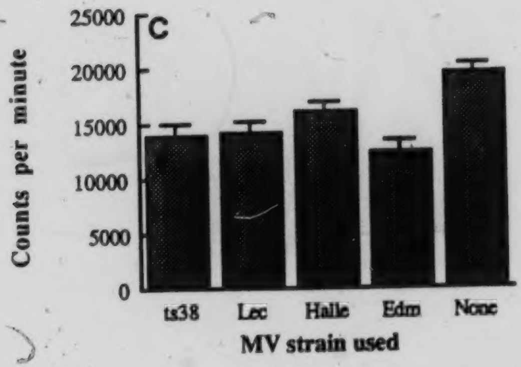
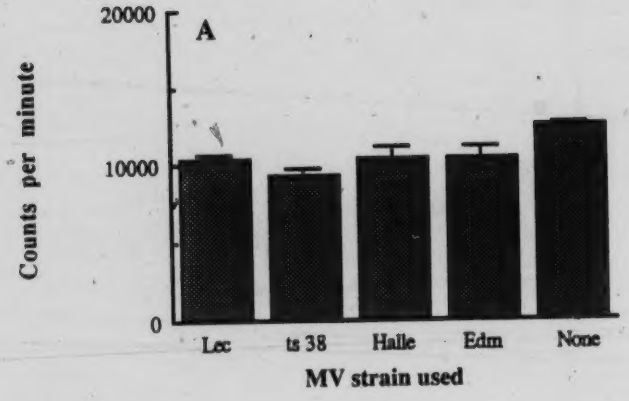

C
Counts per minute
25000
20000
15000
10000
5000
0
ts38
Lec
Halle
Edm
None
MV strain used

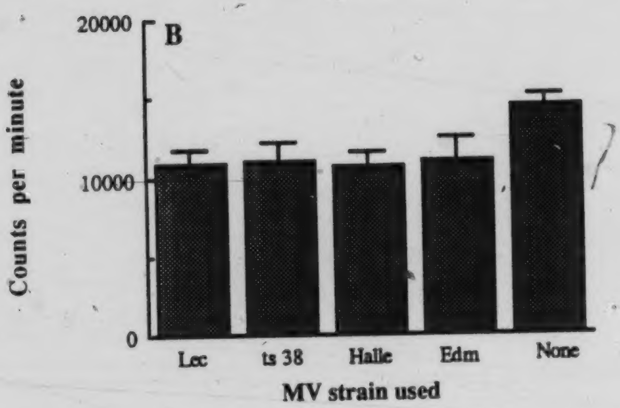
Figure 47. Effect of supernatant solutions (SN) harvested at different times on the growth of HEp-2 cells in vitro. SN were harvested from PHA-stimulated PBMC infected with different strains of MV (MV ts38: ts38; MV Lec: Lec; MV Halle: Halle; MV Edmonston: Edm; uninfected cells: None). SN were then UV-irradiated and ultracentrifuged as described in materials and methods. Tests for infectivity of these SN were negative. One hundred µL of SN were added to confluent monolayers of HEp-2 cells, grown for 24 h in 96-well plates (25,000 cells/well) as described in materials and methods. [^{3}H]-thymidine was added for the last 6 h of a total incubation period of 48 h. The cells were then lifted off the surface of the plates by trypsinization and harvested as described in materials and methods. The amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. Each point represents the mean and standard deviation of six parallel cultures.

A: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 24 h of incubation.

B: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 48 h of incubation.

C: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 72 h of incubation.


A
Counts per minute
20000
10000
0
Lec
ts 38
Halle
Edm
None
MV strain used


B
Counts per minute
20000
10000
0
Lec
ts 38
Halle
Edm
None
MV strain used

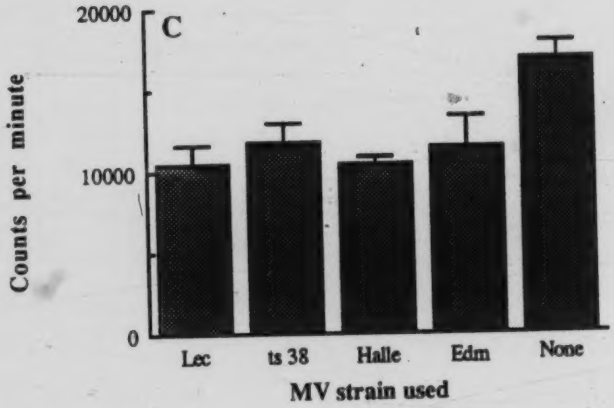
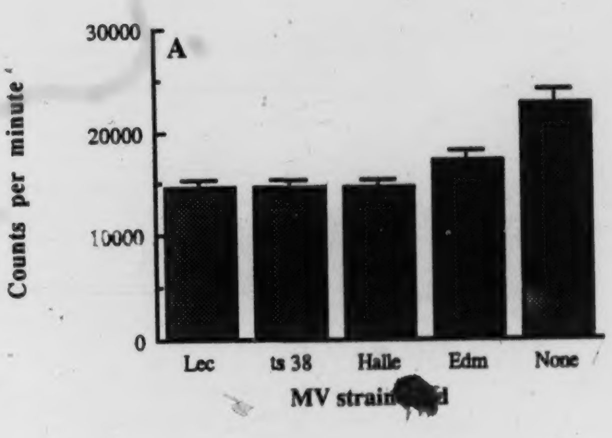

C
Counts per minute
20000
10000
0
Lec
ts 38
Halle
Edm
None
MV strain used

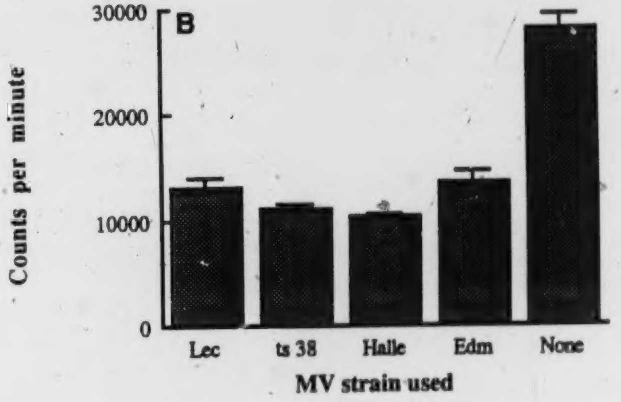
Figure 48. Effect of supernatant solutions (SN) harvested at different times on the growth of Hela cells in vitro. SN were harvested from PHA-stimulated PBMC infected with different strains of MV (MV ts38: ts38; MV Lec: Lec; MV Halle: Halle; MV Edmonston: Edm; uninfected cells: None). SN were then UV-irradiated and ultracentrifuged as described in materials and methods. Tests for infectivity of these SN were negative. One hundred µL of SN were added to confluent monolayers of Hela cells, grown for 24 h in 96-well plates (25,000 cells/well) as described in materials and methods. [^{3}H]-thymidine was added for the last 6 h of a total incubation period of 48 h. The cells were then lifted off the surface of the plates by trypsinization and harvested as described in materials and methods. The amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. Each column represents the mean and standard deviation of six parallel cultures.

A: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 24 h of incubation.

B: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 48 h of incubation.

C: Addition of SN obtained from MV-infected, PHA-stimulated PBMC harvested after 72 h of incubation.


A
Counts per minute
30000
20000
10000
0
Lec
ts 38
Halle
Edm
None
MV strain [illegible]d


B
Counts per minute
30000
20000
10000
0
Lec
ts 38
Halle
Edm
None
MV strain used

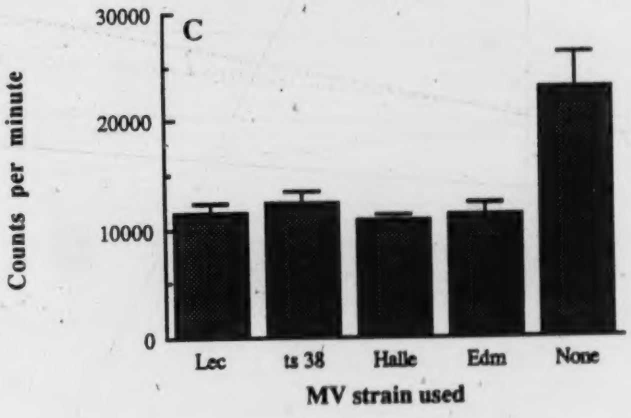

C
Counts per minute
30000
20000
10000
0
Lec
ts 38
Halle
Edm
None
MV strain used

Figure 49. Effect of different dilutions of MV-infected PBMC supernatant solutions (SN) on the growth of HeLa cells in vitro. SN were harvested from PHA-stimulated PBMC that were infected with different strains of MV. SN were then UV-irradiated and ultracentrifuged as described in materials and methods. Tests for infectivity of these SN were negative. One hundred μL of SN were added to confluent monolayers of Hela cells, grown for 24 h in 96-well plates (25,000 cells/well) as described in materials and methods. $[^3H]$-thymidine was added for the last 6 h of a total incubation period of 48 h. The cells were then lifted off the surface of the plates by trypsinization and harvested as described in materials and methods. The amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. Each column represents the mean and standard deviation of six parallel cultures.

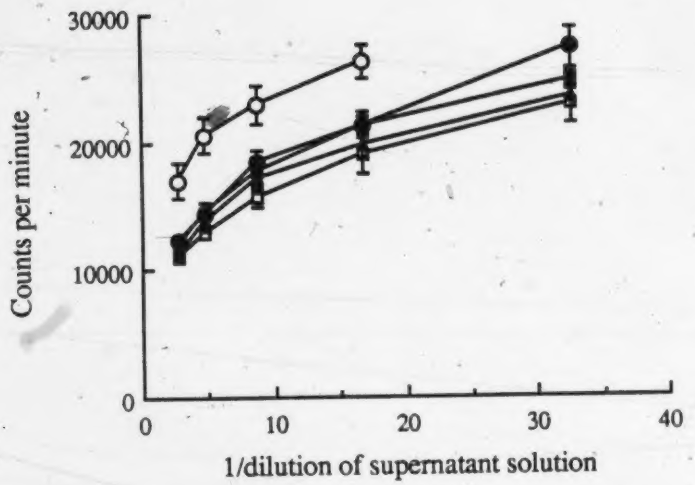
(○) Addition of SN from uninfected, PHA-stimulated PBMC

(●) Addition of SN from MV Edmonston-infected, PHA-stimulated PBMC

(■) Addition of SN from MV ts38-infected, PHA-stimulated PBMC

(▲) Addition of SN from MV Lec-infected, PHA-stimulated PBMC

(▫) Addition of SN from MV Halle-infected, PHA-s stimulated PBMC


Counts per minute
30000
20000
10000
0
0
10
20
30
40
1/dilution of supernatant solution

synthesis in HeLa cells. The results, shown in Table 7, indicated that the inhibitory effect was not significantly different at 37°C compared to 32°C, for both MV ts38 and MV Lec. These data suggested that the temperature sensitivity of MV ts38 did not affect the ability of the virus to induce the synthesis of DNA inhibitory molecules.

3. Presence of IFNs in supernatant solutions and its effect on HeLa cell growth

Our previous results indicated that IFNα was one of the mediators of MV-induced abrogation of PBMC proliferation. To assess the role of IFNα in the SN ability to interfere with the DNA synthesis of HeLa cells, the amount of IFNα present in the SN was determined and 200 to 400 IU/mL was found. Treatment of the SN with 4500 NU/mL of anti-IFNα resulted in the disappearance of all of the antiviral activity. SN treated with different amounts of anti-IFNα were tested on HeLa cell growth and the results are shown in Table 8. In the presence of 2250 NU/mL of anti-IFNα, some reversal of the inhibitory effect was observed. And the reversal of the inhibitory effect increased, when 4500 NU/mL of anti-IFNα was used. However, no significant change was observed when the amount of anti-IFNα was increased from 4500, up to 18000 NU/mL. Similarly, when equivalent dilutions of control sheep serum were used, no change in the inhibitory effect was observed, irrespective of the dilution used. This indicated .

TABLE 7. Effect of supernatant solutions obtained from MV Lec or MV ts38-infected PBMC grown at 32°C or 37°C on the growth of HeLa cells in vitro.

Virus	Counts per minute	
	37°C	32°C
MV ts38	43346 ± 3422	46301 ± 2886
MV Lec	47391 ± 2501	41046 ± 2374
None	85513 ± 3422	87362 ± 4220

MV ts38 or MV Lec-infected or uninfected PBMC were incubated at 32°C or 37°C in the presence of 10 µg/mL of PHA. SN were harvested 48 h later. The SN were UV-irradiated, ultracentrifuged and tested on Hela cells grown in 96-well plates for 24 h, as described in materials and methods. [^{3}H]-thymidine was added for the last 6 h of a total incubation period of 48 h. The cells were lifted off the surface of the plates by trypsinization and the amount of radioactivity incorporated was determined. The results are expressed as the amount of radioactivity incorporated. Mean and standard deviation of six parallel cultures are shown for each set of data.

Table 8. Effect of anti-IFNα on the inhibitory effect of supernatant solutions harvested from MV ts38-infected PBMC.

Amount of anti-IFNα used/ml	Counts per minute	Dilution of control serum	Counts per minute
0	54189 ± 2137	0	54189 ± 2137
2250	62426 ± 1874	1/200	50170 ± 1253
4500	68356 ± 2604	1/100	51242 ± 4578
9000	70993 ± 3230	1/50	51378 ± 2998
18000	70448 ± 2570	1/25	54639 ± 2508

SN were harvested from 48 h cultures of MV ts38-infected and PHA-stimulated PBMC. The SN were mixed with either different amounts of anti-IFNα or with the same dilutions of control serum. The different mixtures were incubated at 37°C for one hour and then added to Hela cells that were seeded 24 h earlier in 96-well plates. [^{3}H]-thymidine was then added for the last 6 h of a total incubation period of 48 h. The cells were then lifted off the surface of the plates by trypsinization and the amount of radioactivity incorporated was determined. The results are expressed as the amount of radioactivity incorporated. Each set of data represents the mean and standard deviation of six parallel cultures.

CPM in absence of supernatant solutions : 74133 ± 2040 that the observed effect of anti-IFNα was not a property of the serum, but rather of the antibody.

To determine if IFNγ was involved in the inhibitory effect, the SN was treated at pH 2.0 for 48 h as indicated in materials and methods. Treatment of the SN at pH 2.0 for 48 h did not affect the inhibitory effect of the SN, indicating that IFNγ was not involved. (Table 9).

4. Effect of viral proteins as DNA-synthesis inhibitors

Polyclonal antibodies against MV were used to neutralise viral proteins present in the SN. The SN was first centrifuged at 100,000 x g, then incubated with either MV antiserum or a control serum and centrifuged again at 100,000 x g to remove any immune complexes formed. No significant difference was observed between the treated SN and the untreated ones with respect to their inhibitory potential on the growth of HeLa cells (Table 10), indicating that MV proteins were not inhibitory in this test system.

5. Role of enriched monocyte and enriched T cell populations in the production of DNA-synthesis inhibitors

Our earlier results indicated that enriched monocyte populations were the most important cells in blocking PBMC

Table 9. Effect of supernatant solutions of MV ts38-infected and PHA-stimulated PBMC treated at pH 2.0, on the growth of HeLa cells *in vitro*.

	Supernatant solutions from		
Dilution	Virus-infected PBMC	Virus-infected PBMC + pH 2.0 treatment	Uninfected PBMC
1:2	62474 + 2051*	60774 + 1788	81584 + 2049
1:4	66789 + 2855	67492 + 3586	76170 + 2713

* Counts per minute

SN were harvested from MV ts38-infected or uninfected PBMC, UV-irradiated, ultracentrifuged and dialysed against RPMI adjusted to pH 2.0 for 48 h. The SN were then dialysed against RPMI at pH 7.5 for another 48 h. SN at either 1:2 or 1:4 dilutions were added to HeLa cells seeded 24 h earlier as described in materials and methods. [^{3}H]-thymidine was added for the last 6 h of a total incubation period of 48 h. The cells were then lifted off the surface of the plates, harvested as described earlier and the amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. The data shown represent the mean and standard deviation of six parallel cultures.

Table 10. Effect of MV antibodies on the inhibitory effect of supernatant solutions obtained from MV-infected, PHA stimulated PBMC at 37°C.

Treatment	Supernatant solutions from	
	Virus-infected PBMC	uninfected PBMC
MV antiserum	56545 ± 3859*	105665 ± 1781
Normal rabbit serum	61594 ± 2607	101840 ± 3133
None	51471 ± 5433	105580 ± 1463

* Counts per minute

MV ts38-infected and PHA stimulated PBMC cultures were incubated at 37°C for 48 h and the SN were harvested. The SN were UV-irradiated, ultracentrifuged and incubated with either MV polyclonal antiserum or normal rabbit serum, both used at a 1:50 dilution. The different mixtures were incubated at 37°C for one hour. Ultracentrifugation was carried out as described in materials and methods. The SN were then added to HeLa cells seeded 24 h earlier in 96-well plates. [^{3}H]-thymidine was added for the last 6 h of a total incubation time of 48 h. The cells were then lifted off the surface of the plates and the amount of incorporated radioactivity determined. The results are expressed as the amount of incorporated radioactivity. Each set of data represents the mean and standard deviation of six parallel cultures.

proliferation *in vitro*. Experiments were designed to determine the importance of the different subsets of cells in relation to the inhibitory effect of the SN on HeLa cell growth. Enriched T cell, enriched monocyte populations and PBMC were infected with MV ts38, stimulated with PHA or LPS, and SN harvested at 48 h, p.i. The SN were then tested in the HeLa cell assay as described earlier. SN obtained from MV ts8-infected PBMC and enriched monocyte populations were inhibitory whereas SN obtained from MV ts38-infected enriched T cell populations were not (Table 11). The inhibition resulting from SN obtained from MV ts38-infected enriched monocyte populations was higher than that obtained from infected PBMC. These results, similar to the earlier observations, indicated that monocytes played an important role in the inhibitory effect.

TABLE 11. Effect of supernatant solutions obtained from MV ts38-infected T cell-enriched populations, monocyte-enriched populations and PBMC on the growth of HeLa cells.

Cells	Counts per minute	
	Infected	Uninfected
PBMC	59733+1273	73749+2575
T Cells	68234+869	68947+1273
Monocytes	43869+1532	58845+1364

T cell-enriched populations, monocyte-enriched populations and PBMC were infected at an m.o.i. of 5. The cells were then incubated at 37°C in the presence of 10 µg/mL of PHA for 48 h. The SN were harvested, UV-irradiated and added to Hela cells that were seeded 24 h earlier. [^{3}H]-thymidine was added for the last 6 h of a total incubation time of 48 h. The cells were then lifted off the surface of the plates by trypsinization and the amount of incorporated radioactivity was determined. The results are expressed as the amount of incorporated radioactivity. Each set of data represents the mean and standard deviation of six parallel cultures.

DISCUSSION

A. Replication of MV ts38: retention of temperature sensitivity in PBMC and induction of an abortive infection.

In the present study, the interaction of a ts mutant of measles virus, MV ts38, with PBMC was investigated. MV ts38 did not go through a full cycle of virus replication at 37°C or 39°C in PBMC as well as in population of cells enriched for T cells or monocytes. Viral mRNAs and viral proteins were detected in all three cell populations at all temperatures examined. At the permissive temperature, however, MV ts38 replicated more efficiently in enriched T cell populations than in enriched monocyte populations (Figures 34 and 35). At 37°C, MV ts38 interfered with PBMC proliferation, without killing the cells (Figure 44). Enriched monocyte populations were found to play an important role in the inhibitory effect. One of the mediators of the inhibitory effect was found to be IFNα.

In the studies on MV replication in PBMC, the results indicate that in MV Lec-infected PBMC, infectious particles were assembled and released at all temperatures tested, but in higher numbers at 32°C (Figure 3). This could be due to the generation of defective interfering particles at the higher temperatures (Tsang *et al.*, 1981; Holland, 1980). In MV ts38-infected PBMC, large numbers of infectious particles were detected at all times post infection, at 32°C. At 37°C and 39°C, on the other hand, infectious virus was detected for the first 24 h post infection (Figure 4). While the test used did not differentiate between progeny virus and infectious particles remaining from the inoculum, the kinetics of decay of the virus in medium alone (Figure 6) would suggest that the infectious particles detected were most likely remnants of the inoculum virus. On that basis, it can be concluded that MV ts38 did not go through a full cycle of virus replication at 37°C and 39°C.

It can also be concluded that PBMC stimulation was required for MV replication in these cells, as a rapid decrease in infectious virus was observed in unstimulated PBMC (Figures 3 and 4). It has been previously reported that MV replication takes place only in stimulated PBMC (Sullivan *et al.*, 1975; Hyypiä *et al.*, 1985), though it has also been reported that a low level of replication occurs in unstimulated PBMC (Joseph *et al.*, 1975). However, it is well established that stimulation of PBMC is required for efficient replication of MV to take place (Joseph *et al.*, 1975; Sullivan *et al.*, 1975; Hyypiä *et al.*, 1985) and the results obtained in this study are in agreement with these conclusions.

In MV ts38-infected Vero cells, a large amount of infectious virus was detected at 32°C. At 37°C and 39°C, infectious particles were present at the different times tested, but to a much lesser extent, and in amounts decreasing with time, suggesting that the infectious particles were remnants of the inoculum (Figure 2). These conclusions were confirmed in that no mRNA or viral protein was detected at these temperatures in MV ts38-infected Vero cells (Figures 15 to 22). In contrast, MV Lec replication in Vero cells resulted in the release of infectious particles at all three temperatures tested, with 32°C giving the highest virus titer (Figure 1). More efficient MV replication at a lower temperature has been reported earlier in the literature (Lucas *et al.*, 1978; Tsang *et al.*, 1981), and growing the virus at a lower temperature is considered the best way to produce a high titer of infectious particles (Scott *et al.*, 1982).

The plaquing efficiency (plaque titer at 32°C/plaque titer at 37°C or 39°C) of MV ts38 in PBMC was greater than one thousand at 30 h post infection (Figure 4). Taking into account the replication time of the virus and the rate of decay of the input virus, it was logical to observe the plaque efficiency after a lag period, i.e, 30 h. MV ts38 would be classified as a ts mutant in PBMC, on the basis of the plaquing efficiency. Temperature sensitive mutants have been defined as "viruses that replicate well at low temperatures (permissive) and poorly at higher temperatures (restrictive)" (Richmond *et al.*, 1979) and according to that definition, both MV ts38 and the parental strain would be considered temperature sensitive. However, a certain consensus has emerged in the literature over the years that a plaquing efficiency of more than a hundred is required for a virus to be considered temperature sensitive (Sandford *et* *al*., 1988; Minagawa *et al*., 1976; Ju *et al*., 1976; Haspel *et al*., 1975; Preble *et al*., 1973). A more rigorous standard has been set by some workers, using a plaquing efficiency of more than a thousand to classify a mutant as being temperature sensitive (Martin *et al*., 1988; McKay *et al*., 1988; Chui *et al*., 1986; Bergholz *et al*., 1975). With a plaquing efficiency of more than a thousand, MV ts38 satisfies the most rigorous standards used to define temperature sensitivity of a virus.

Hybridization studies indicate that transcription occurred at a normal rate in MV ts38-infected PBMC as mRNA in both MV ts38 and MV Lec-infected PBMC were detected at the same time post infection at all three temperatures tested (Figures 11 to 13). The sizes of the mRNAs were also similar, indicating that there was no defect in transcription. In Vero cells, on the other hand, mRNA was not detected at 48 h post infection, at 37°C or 39°C, whereas in MV Lec-infected Vero cells, the messages were present in detectable amounts, at these same temperatures (Figures 15 and 16). These results indicate that transcription was blocked in MV ts38-infected Vero cells at the restrictive temperatures. At 37°C, detectable levels of mRNAs were seen at 4 days p.i, and at 39°C at 6 days post infection. These observations indicate that MV ts38 transcriptional activity in Vero cells was taking place at a slower rate at the restrictive temperatures, decreasing with increasing temperature. It can also be inferred that there are at least two blocks in MV ts38 transcription, giving the mutant the temperature sensitive characteristics. One at the level of transcription, operating in Vero cells, keeping transcriptional activity to a minimum and which is not observed in PBMC. However, MV ts38 is also temperature sensitive in PBMC, as indicated by plaque assay results, implying the presence of a second block, the latter being manifest only in PBMC. The second block is probably found at a late stage of virus replication. The presence of at least two mutations renders the mutant stable, a rare occurrence in RNA viruses due to the high rate of mutation (Ramig, 1985).

The immunofluorescence studies (Figures 17 to 22) indicate that no viral protein synthesis took place in MV ts38-infected Vero cells at the restrictive temperatures, an expected result in light of the lack of viral mRNA. At these same temperatures, viral protein synthesis took place in MV Lec-infected Vero cells, clearly indicating that the absence of viral proteins in MV ts38-infected Vero cells was an inherent property of the mutant and not the cell line nor the techniques and reagents used. In contrast, the immunofluorescence results revealed that viral protein synthesis took place in both MV Lec and MV ts38-infected PBMC at all three temperatures tested. Positive results were obtained in MV ts38-infected PBMC for the presence of all the major viral proteins tested. These results, and those obtained from the hybridization studies mean that the MV ts38 genome is transcribed and translated in PBMC. The hybridization studies also show that replication of the genome is not blocked as there is accumulation of genomic RNA within these cells (Figure 14). From these results, it can be inferred that the block in MV ts38 replication at the restrictive temperatures in PBMC is at the level of maturation or release.

The properties shown by MV ts38 upon infecting PBMC at 37°C are those of an abortive infection. An abortive infection is one that does not synthesize any infectious particle even though some or all of the viral proteins may be synthesized (White *et al.*, 1987), i.e, the properties shown by MV ts38-infected PBMC at the restrictive temperatures. Persistent infections also fulfill the same criteria. Viral antigens are present in most persistently infected cells (Rustigian, 1966a; Norrby, 1967; Gould *et al.*, 1975) as in MV ts38-infected PBMC. While most of the persistently infected cells yield small amounts of virus (Minagawa *et al.*, 1976; Jacobson *et al.*, 1982; Gould *et al.*, 1975), there are examples of non yielding cultures containing viral antigens (Rustigian *et al.*, 1966b; Wilson *et al.*, 1973) as is the case in MV ts38-infected PBMC. While it has been suggested that the generation of temperature sensitive mutants might be a way in which viruses could establish persistent infection (Preble *et al.*, 1973) and that abortive infections can lead to persistent ones (Roizman, 1985), the duration of the experiments preclude a definitive answer as to whether MV ts38 can establish a long term persistent infection in PBMC.

The interaction of MV ts38 with PBMC at the restricted temperature can be considered to be an "immediate persistent infection" (Wechsler *et al.*, 1987). A persistent infection *in vivo* must satisfy two criteria: firstly, the virus must not be cytopathic for the host cell it infects; and secondly, the infected cell must escape recognition by the host immune system that can eliminate it (Oldstone, 1984). *In vitro*, however, only the first criterion needs to be satisfied. A persistent infection *in vitro* generally occurs following an acute lytic infection in which most of the cells are destroyed (Wechsler *et al.*, 1987; Rima *et al.*, 1976). The few surviving cells will grow, resulting in a persistent infection. The fact that few surviving cells are observed leads to the possibility that an aberrant cell is being recloned and studied. When virus mutants are used, non lytic infections may occur, i.e, the cells survive the initial infection and are immediately available for study. Under these conditions of virus-cell interactions, the term "immediate persistent infection" has been used (Wechsler *et al.*, 1987). In the present study, a temperature sensitive mutant has been used to infect PBMC. At the restrictive temperature, all the cells survive the infection, satisfying the only criterion for persistency *in vitro*. However, taking into account the duration of the experiments described, the term "immediate persistent infection" would be more appropriate.

B. Inhibitory effect of MV abortive infection on PBMC stimulation and its implications.

Decreased proliferation was observed in MV ts38-infected PBMC upon stimulation by PHA (Figure 24). The decrease in PBMC proliferation was smaller than when other strains from SSPE patients such as MV Halle and MV Lec were used. However, similar decreases were observed when wild-type laboratory strains such as Edmonston or VSC were used. The results indicate that MV ts38 inhibitory effect is within the range of inhibition exerted by commonly used laboratory strains. These experiments were carried out at 37°C, a temperature at which MV ts38 did not undergo a full cycle of virus replication. Thus it can be concluded that a full cycle of virus replication is not required for MV to interfere with PBMC proliferation. Similarly, when other stimuli, such as HSV antigens, anti-OKT3 antibodies or mixed lymphocyte reactions, were used for PBMC proliferation there was a decrease in proliferation (Figures 25 to 27). However, under these conditions, no difference was observed between the extent of inhibition of MV ts38 and that of MV Lec. PHA is a strong T cell mitogen triggering the proliferation of more T cells than any of the other stimuli used, as indicated by the amount of radioactivity incorporated. Thus, any small difference in the extent of interference by the two viruses would be amplified by a strong stimulus and not necessarily detected when less powerful stimuli were used.

The results stress the fact that while all MV strains used were inhibitory, the extent of that inhibitory effect differs. It is worth noting that work with lymphocytic choriomeningitis virus (LCMV) infection in mice has shown that some genetic variants of LCMV generated within infected mice were suppressive while others were not (Ahmed *et al.*, 1984). It could be speculated that infection by MV would lead to genetic variants within the body, some more suppressive than others, thus explaining the differences observed in individuals during the clinical phase of the disease (Walsh, 1983; Coovadia *et al.*, 1978). In fact, it has been shown that complications following measles could be predicted on the basis of the severity of the suppression (Coovadia *et al.*, 1978). Moreover, genomic variants of MV, differing in several nucleotides, are produced during persistent infections (Cattaneo *et al.*, 1988). A variation of 30 to 40 nucleotides within the approximately 16,000 nucleotides of the virus was noted when the genomes of two variants, isolated during a persistent infection, were examined. The generation of MV variants has been suggested to be important in the pathogenesis of the disease (Hamilton *et al.*, 1973). The generation of variants within RNA viruses is a natural occurrence. The rate of spontaneous mutations in RNA viruses are of the order of 10^{-3} to 10^{-4} per incorporated nucleotide due to low fidelity of RNA replication, resulting from the lack of proofreading activities within the RNA replicating enzymes (Ramig, 1985; Holland *et al.*, 1982).

C. Mechanism(s) by which MV interferes with PBMC proliferation

1. Importance of monocytes in the inhibitory effect of MV

From the results obtained with enriched cell populations, it can be concluded that monocytes are the most important cells in bringing about MV abrogation of PBMC proliferation (Figures 29, 31 and 32). These results are in accordance with recent published reports (Salonen *et al.*, 1989; Griffith *et al.*, 1987) and differ from an earlier one (Lucas *et al.*, 1978). The apparent contradiction could be due to different depletion techniques employed. While Lucas and coworkers used only the adherence properties of monocytes for enrichment, the results shown in this work were obtained from cell populations that were purified by adherence followed by the killing of the undesired cell populations with specific antibodies and complement. Furthermore, a second technique was used for the enrichment of each cell population: for T cells, the non adherent cells were further purified by passage through nylon wool and for monocytes, a percoll discontinuous density gradient was used. The percentage of purity obtained from these techniques was above 90% as deduced by immunofluorescence using monoclonal antibodies against specific cell markers. Concordant results were obtained from cell populations isolated by both techniques. It must be pointed out that in all the enriched cell populations used, a small number of contaminating cells was always present. However, increasing the amount of infected cells from monocyte-enriched populations increased the inhibitory effect whereas increasing amounts of infected cells from T cell-enriched populations did not affect the proliferation of PHA stimulated PBMC, reinforcing the role of monocytes in the inhibitory effect. Also, the fact that a different strain of MV was used by Lucas and coworkers (Lucas et al., 1978) could be another source of variation. However, Salonen and coworkers (Salonen *et al.*, 1989), using a different strain of measles virus and Griffith and coworkers (Griffith *et al.*, 1987), using blood from patients suffering from measles, concluded that monocytes were of importance in the inhibitory effect of the virus. And in this study, the same conclusions were reached with a ts mutant that does not undergo a full cycle of replication at 37°C, the temperature at which these experiments were conducted.

2. Monocyte ageing is of no relevance to the inhibitory effect exerted by MV

It is well established that incubation of monocytes *in vitro* leads to the maturation of these cells into macrophages (Rinehart *et al.*, 1979; Mayernick *et al.*, 1984).

The results (Figure 33) indicate that incubation of enriched monocyte populations *in vitro* did not affect MV ts38 inhibitory effect. These results suggest that the maturation of enriched monocyte populations *in vitro* is of no relevance with respect to the inhibitory effect of the virus.

3. Importance of MV cytopathic effect varies depending on the strain used

Results of direct cell counts at 37°C (Figure 44) showed that the percentage of live cells in cultures of MV ts38-infected and stimulated PBMC was similar to uninfected stimulated cultures, implying that MV ts38 did not kill any cell. These observations suggest that cell death was not required for MV to inhibit PBMC proliferation. However, with the parental strain at all three temperatures and MV ts38-infected PBMC at 32°C, a significant amount of cell death was observed. Thus, while it can be deduced that cell death is not required for the abrogation of PBMC proliferation by MV ts38, the possibility that cell death contributes to the inhibitory effect when other strains of MV are used, cannot be argued against. It has previously been suggested that cell death induced by MV may be responsible for the suppression observed (Borysiewick *et al.*, 1985) and earlier work has shown that cell death does contribute to the inhibitory effect at a late stage of MV infection *in vitro* (Salonen *et al.*, 1989). This observation is in accordance with the clinical picture, where a decrease in total cell counts was observed during the disease (Griffith *et al.*, 1986; Alpert *et al.*, 1984). By not killing the infected cells, and still being immunosuppressive, means that in an *in vivo* situation, the virus genome within cells could be carried to distant sites before being cleared by the immune system. In fact, MV genomes have been detected in different organs of the body both during and years after the disease (Moench *et al.*, 1988; Fournier *et al.*, 1988; Fournier *et al.*, 1985). Also, the fact that there was no cell death attributed to MV ts38 indicates that there was no cell fusion and hence no spread of the MV genome by fusion from within, an important aspect of MV dissemination to be considered when the importance of different cell populations are being assessed. One advantage of using MV ts38 in such studies, is that the infection remains limited to the original group of cells infected, i.e, there was no cross contamination, a likely but unaccounted for occurence in most studies and a possible explanation for result discrepancies (Salonen *et al.*, 1989; Griffith *et al.*, 1987; Lucas *et al.*, 1978).

The inability of MV ts38 to kill cells indicated a lack of fusogenic properties by the virus at 37°C. However, adsorption was carried out at 37°C for all experiments performed. The fact that adsorption was carried out at a restrictive temperature and that viral proteins were synthesized at that same restrictive temperature means that MV ts38 entered the cell, in spite of an apparent lack of fusion activity. A lack of fusion activity could be due to uncleaved Fo protein (Fujinami *et al.*, 1981), an unlikely explanation in MV ts38-infected PBMC as F_1, a product of cleaved Fo, is detected at 37°C. However, minor differences in cleavage affecting biological properties but not molecular weight, is a possibility. MV ts38 might have entered the cells by receptor mediated endocytosis, as do some other enveloped animal viruses (White *et al.*, 1983; Wiley *et al.*, 1985). The presence of a putative MV receptor on Vero cells has been documented (Krah *et al.*, 1988). Alternatively, MV ts38 entered the cell by fusion and yet the F protein synthesized within the cells did not induce fusion, a phenomenon which has been described in cells persistently infected with parainfluenza virus 3 (Wechsler *et al.*, 1987) and MV (Wild *et al.*, 1978; 1979). One possible explanation would be an alteration in the lipid composition of the infected cell membrane rendering it resistant to fusion. The alteration could be a response of the infected cells to the presence of viral proteins or a direct result of the presence of viral proteins in the membrane.

4. More efficient replication of MV in enriched T cell populations than in enriched monocyte populations

The importance of monocytes, once established, raised the possibility that there could be preferential replication of MV ts38 in monocytes. The results of these experiments showed that, at 37°C, a full cycle of viral replication does not occur in either enriched monocyte populations or enriched T cell populations, and at the permissive temperature, there was more efficient replication of MV ts38 in enriched T cell populations than in enriched monocyte populations (Figures 34 and 35). More efficient replication of MV Lec was also found in enriched T cell populations at all three temperatures tested, thus ruling out the possibility that MV ts38 behaviour could be an aberrant one (Figures 36 and 37). It has also been reported earlier that MV replication is more efficient in T cells than in monocytes (Joseph *et al.*, 1975). Although more infectious virus was released from T cells than from monocytes, no significant difference was observed in the amount of viral RNA present in either cell types. Furthermore, no significant difference was observed in the amounts of N and H proteins in enriched monocyte populations compared to enriched T cell populations (Table 4). One possible explanation for the presence of similar amounts of viral proteins in the two populations of cells and significantly less infectious virus in enriched monocyte populations would be the degradation of the viral proteins by hydrolysing enzymes, found in large amounts in these cells (Roitt, 1985). However, the presence of full size viral proteins in enriched monocyte populations as well as the release of infectious virus at the permissive temperature would suggest that only some of the viral proteins synthesized are degraded.

5. IL-2 level is not affected by MV infection.

The level of IL-2 was not affected by MV ts38 infection (Figure 45), indicating that the virus did not interfere with the secretion of IL-2, nor does the virus inactivate it. These results are in accordance with what has been published (Borysiewick *et al.*, 1985; Salonen *et al.*, 1988). It has also been previously shown that the ability to respond to IL-2 is not affected by infection of PBMC with MV (Borysiewick *et al.*, 1985) up to 30 h post infection, implying that the IL-2 receptors are not affected by the virus.

6. IFNα plays an important role in the inhibitory effect exerted by MV

IFNα has been found to be involved in the inhibitory effect of MV ts38 on PBMC proliferation. The level of IFNα in MV ts38-infected PBMC cultures was found to be within 100 to 500 IU/mL. The variation in the amount of IFNα detected was most likely due to the source of blood used. Different donors secrete different levels of IFNα. Similar results have been obtained with other strains of MV (Sanchez *et al.*, 1988; Ilonen *et al.*, 1988). However, Lucas *et al.* (1978) have failed to detect any IFN in MV-infected PBMC, while Jacobson *et al.* (1982) have detected much larger amounts.

In the presence of anti-IFNα, partial reversal of the inhibitory effect was observed implying that IFNα is partially responsible for the inhibitory effect observed (Table 5). It is an established fact that IFNα does block the proliferation of PBMC *in vitro* (Gresser, 1977; Einhorn *et al.*, 1983; Francois *et al.*, 1988). The role of monocytes in the inhibitory process and that of IFNα in the same process reinforce each other as monocytes are the main producers of IFNα (Saksela *et al.*, 1984). It is also known that T cells, upon stimulation produce IFNγ (Trinchieri *et al.*, 1985). However, for the IFN assays, bovine cells, which are not responsive to IFNγ were used (Lebon *et al.*, 1979; Trincheri *et al.*, 1985). As well, the antiserum used was directed mainly against IFNα and to a much lesser extent against IFNβ, but not IFNγ.

Conflicting reports have been published with respect to the role of IFNα in MV-induced suppression (Salonen *et al.*, 1989; Sanchez-Lanier *et al.*, 1988; Neighbour *et al.*, 1979; Lucas *et al.*, 1978). A role for IFNα has been found in some of these studies (Salonen *et al.*, 1989; Neighbour *et al.*, 1979) while others have failed to detect any (Sanchez-Lanier *et al.*, 1988; Lucas *et al.*, 1978). One source of variation could be the strain of MV used. It has been shown that the level of IFNα secreted differs depending on the strain used (Ilonen *et al.*, 1986; Volckaert-Vervliet *et al.*, 1978). It has also been reported that IFNα is a mediator of the inhibitory effect of MV Edmonston on PBMC proliferation but not that of MV Halle under similar conditions (Salonen *et al.*, 1989). Taken together, these findings mean that the role of IFNα in MV-induced inhibitory effect varies depending upon the strain used. The results also indicate that MV can induce IFNα production without undergoing a full cycle of virus replication. While the same phenomenon has been observed for some other viruses, e.g, HSV and poliovirus (Lebon *et al.*, 1982; Capobianchi *et al.*, 1985; Pitkäranta *et al.*, 1988), it has never before been reported for MV.

7. MV ts38 infection is a prerequisite for the inhibition of PBMC proliferation

MV ts38 production of proteins in PBMC raised the possibility of MV proteins being inhibitory. The results obtained when UV-inactivated MV ts38 was used (Table 6) indicate that the amounts of MV proteins used in the experiments are not inhibitory, confirming previously published reports (Lucas *et al.*, 1977; Sullivan *et al.*, 1975). These results suggest that an infection is required for MV to inhibit PBMC proliferation; and as discussed earlier, a full cycle of virus replication is not required for the inhibitory effect to be exerted by the virus. Taken together, these findings suggest that the virus effect on PBMC takes place between uncoating of the viral genome and viral protein synthesis in the replication cycle of the virus. Though the results presented showed that MV proteins are not inhibitory when added exogeneously, the possibility that endogeneous viral proteins may be important in the inhibitory effect cannot be excluded. However, the fact that monocytes are the most important cells involved in the inhibitory effect of MV, and that these cells are phagocytic (Roitt, 1985), suggest that some MV proteins will be phagocytosed. The conclusion would be that the presence of MV proteins within the cells, i.e, endogeneous viral proteins, is of no relevance to the suppressive effect of the virus. Thus the stage in the replication cycle of MV that might be of relevance to the inhibitory effect is most likely found after uncoating and before or during protein synthesis. It is known that the mere presence of viral genomes within cells might be sufficient to cause a certain imbalance in the normal activity of the infected cells (Oldstone, 1986; 1984). However, the amount of viral proteins added in the form of inactivated virus is much less than the amount of viral proteins that would be present following an infection. Thus it would be more accurate to state that viral proteins in similar amounts to the infectious virus inoculum used throughout this work is not inhibitory.

D. Presence of DNA-synthesis inhibitory molecules in the supernatant solutions of MV-infected PBMC

1. Effect on HeLa, Vero and HEp-2 cells

The presence and identification of suppressive factors in the SN was determined by using continuous cell lines. Continuous cell lines were used as it was difficult to obtain one constant source of blood for the generation and testing of SN. Three cell lines were tested and while all three were sensitive to the inhibitory molecules present within the SN, HeLa cells were the most responsive (Figures 46 to 48). However, the differences observed between the different strains in the abrogation of PHA stimulated PBMC response was not observed when the SN obtained from these same strains were tested on HeLa cells. These results suggest that the cell lines tested were sensitive to some inhibitory molecules, and not to others within the SN. Alternatively, the difference observed in the inhibitory effect of different MV strains could be due to the cytopathic effect of MV, i.e, the difference could have been due to cell death. If that is so, the difference seen when different strains are used would not be detected when SN were used. And in the HeLa cell test, SN, devoid of any infectious virus, is used; thus eliminating the cytopathic effect of the virus.

2. Effect of temperature, anti-IFNα, MV antibodies and pH 2.0 treatment on the inhibitory molecules.

Partial or complete replication of MV ts38 induced the same amount of suppressive factors as no difference was observed in the extent of the HeLa cell suppression at 32°C compared to 37°C (Table 7). IFNα plays a significant role in the inhibitory effect as anti-IFNα reverses the inhibition observed. IFNγ, on the other hand, does not contribute to the inhibitory effect of the SN as treatment at pH 2.0 left the inhibitory effect intact (Table 9). The results of the experiments point to IFNα as the main, if not the only, inhibitory molecule in this test system.

The removal of viral proteins did not affect the inhibitory effect of the SN (Table 10), indicating that MV proteins do not inhibit DNA synthesis by HeLa cells. These results are in accordance with results obtained when UV-inactivated virus was used. In both cases, no inhibitory activity was attributed to viral proteins which reinforces the point that while an infection is required for the inhibitory factors to be induced, the presence of viral proteins are not.

The inhibitory molecules are synthesized by monocyte-enriched populations more so than by PBMC and not at all by T cell-enriched populations (Table 11). These results confirm previous findings that monocytes were important in the inhibitory effect. Though the cell assay system used might detect some inhibitory molecules and not others, it nevertheless confirms other results presented in this study, attributing to monocytes and IFNα major roles in the inhibitory effect exerted by MV ts38.

E. Overall conclusions

This work describes the interactions of a ts mutant of MV with PBMC at non permissive temperatures. Several important points have emerged from these studies:

1. There are at least two blocks in replication that are responsible for the ts characteristics of MV ts38: An early block prior to transcription operating in Vero cells and a late block operating in PBMC occuring at the level of maturation or release.

2. Under non-permissive temperature, the ts mutant can induce an abortive infection in PBMC. No cell fusion or syncytium formation is observed at the restrictive temperature. An "immediate persistent infection" is established at 37°C by MV ts38 in PBMC. Under such conditions, MV ts38 abrogates PBMC proliferation in vitro; monocyte-enriched populations and IFNα play important roles in the inhibitory effect induced by the virus.

3. More efficient replication of MV ts38 takes place in enriched T cell populations compared to enriched monocyte populations, however, no difference in viral transcription or translation was detected in the two different cell populations.

Such studies are of relevance for several reasons. Measles, as a childhood disease, is not a major health problem in the industrialized world; however, the disease still kills millions of children in the developing world (Walsh, 1983; Marusyk, 1984). Protective vaccines that are able to overcome the interference of maternal antibody has yet to be found (Markowitz et al., 1987). Better understanding of the ways MV interacts with cells might help in the development of better vaccines. The prevalence of ts mutants in nature and the use of such mutants as vaccines means that infections by ts mutants are common occurences and emphasize the importance of understanding the ways ts mutants interact with cells.

It is becoming increasingly evident that many viruses can disrupt cell functions without killing the cells or structurally transforming them. Such observations underline the importance of studies on virus-cell interactions under noncytopathic conditions, a criterion fulfilled in the present study. Furthermore, persistent viral infections have been implicated in many diseases and will likely be implicated in more, giving studies on virus-cell interactions, under these conditions, an important place in contemporary virology.

REFERENCES

Aaby, P. 1988. Malnutrition and overcrowding/intensive exposure in severe measles infection: Review of community studies. Rev. Inf. Dis. 10 : 478-491.

Aaby , P., J. Bukh, J. Leerhoy, I. D. Lisse, C. H. Mordhorst and I. R. Pedersen. 1986. Vaccinated children get milder measles infection: A community study from Guinee-Bissau. J. Inf. Dis. 154 : 858-863.

Adams, J. M. and D. T. Imagawa. 1962. Measles antibodies in multiple sclerosis. Proc. Soc. Exp. Biol. Med. 3 : 562-566.

Anderson, J. F. and J. Golberger. 1911. Experimental measles in the Monkey: A preliminary report. Public Health Rep. 26: 847-856.

Andzhaparidze, O. G., N. M. Chaplygina, N. N. Bogolomolova, V. D. Lotte, I. B. Koptyaeva and Y. S. Boriskin. 1987. Non-infectious morphologically altered nucleocapsids of measles virus from persistently infected cells. Arch. Virol. 95: 17-28.

Ahmed, R., A. Salmi, L. D. Butler, J. M. Chiller and M. B. A. Oldstone. 1984. Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice: role in suppression of cytotoxic T lymphocyte response and viral persistence. J. Exp. Med. 60 : 521-540.

Alkhatib, G. and D. J. Briedis. 1986. The predicted primary structure of the measles virus hemagglutinin. Virology. 150 : 479-490.

Alpert, G., L. Leibovitz and Y. L. Danon. 1984. Analysis of T lymphocyte subsets in measles. 149 : 1018.

Anttonen, O., M. Jokinen, A. Salmi, R. Vainionpää and C. G. Gahmberg. 1980. The glycoproteins of measles virus. Biochem. J. 185 : 189-194.

Arneborn, P. and G. Biberfeld. 1983. T-lymphocyte subpopulations in relation to immunosuppression in measles and varicella. Infect. Immun. 39 : 29-37.

Baczko, K., M. Billeter and V. ter Meulen. 1983. Purification and molecular weight determination of measles virus genomic RNA. J. Gen. Virol. 64 : 1409-1413.

Basle, M. F., W. C. Russell, K. K. A. Goswani, A. Rebel, P. Giraudon, F. Wild and R. Filmon. 1985. Paramyxovirus antigens in Osteoclasts from Paget's bone tissue detected by monoclonal antibodies. J. Gen. Virol. 66 : 2103-2110.

Bellini, W. J., G. Englund, S. Rozenblatt, H. Arnheiter and C. D. Richardson. 1985. Measles virus P gene codes for two proteins. J. Virol. 53908-919.

Bellini, W. J., G. Englund, C. D. Richardson, S. Rozenblatt and R. A. Lazzarini. 1986. Matrix genes of measles virus and canine distemper virus : Cloning, Nucleotide sequences, and deduced amino acid sequences. J. Virol. 58 : 408-416.

Bergholz, C. M., M. P. Kiley and F. E. Payne. 1975. Isolation and characterisation of temperature sensitive mutants of measles virus. J. Virol. 16 : 192-202.

Billeter, W., K. Baczko, A. Schimd and V. ter Meulen. 1984. Cloning of DNA corresponding to four different measles virus genomic regions. Virology. 132 : 147-159.

Blumberg, B. M., G. C. Roux, L. Raju, R. Dowling, P. C. Chollet, and A. Kolakofsky. 1985. Sequence determination of the Sendai virus HN gene and its comparison to the influenza virus glycoproteins. Cell. 41 : 269-278.

Blumberg, B. M., J. C. Crowley, J. I. Silverman, J. Menonna, S. D. Cook and P. C. Dowling. 1988. Measles virus L protein evidences of ancestral RNA polymerase. Virology. 164 : 487-497.

Bohn, W., G. Rutter, H. Honenberg and Mannweiler, K. 1983. Inhibition of measles virus budding by phenothiazines. Virology. 130 : 44-55.

Bohn, W., G. Rutter, H. Honnenberg, K. Hamweiler, K., and P. Nobis. 1986. Involvement of actin filaments in budding of measles virus : studies on cytoskeleton of infected cells. Virology. 149 : 91-106.

Borysiewick, L. K., P. Casali, B. Rogers, S. Morris and J. G. P. Sissons. 1985. The immunosuppressive effect of measles virus on T cell function-failure to affect IL-2 release or cytotoxic T cell activity in vitro. Clin. Exp. Immunol. 59 : 29-36.

Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J. Clin. Lab. Invest. 21 (suppl. 97), 77-88.

Brenan, M. and R. M. Zinkernagel. 1983. Influence of one virus infection on a second concurrent primary in vivo antiviral cytotoxic T cell response. Inf. Immun. 41 : 470-475.

Brown, H. R., N. Goller, H. Thormar and E. Norrby. 1987. Fuzzy material surrounding measles virus nucleocapsids identified as matrix protein. Arch. Virol. 94 : 163-168.

Buckland, R., C. Gerald, R. Barker and T. F. Wild. 1987. Fusion glycoprotein of measles virus : nucleotide sequence of the gene and comparison with other paramyxoviruses. J. Gen. Virol. 68 : 1695-1703.

Burnet, F. M. 1968. Measles as an index of immunological function. Lancet. 2 : 610-613.

Bussell, R. H., D. J. Waters and M. K. Seals. 1974. Measles, canine distemper and respiratory syncytial virions and nucleocapsids. A comparative study of their structure, polypeptide and nucleic acid composition. Med. Microbiol. Immunol. 160 : 105-124.

Capobianchi, M. R., J. Facchini, P. Di Marco, G. Anttonelli and F. Dianzani. 1985. Induction of alpha interferon by membrane intercation between viral surface and peripheral blood mononuclear cells. Proc. Soc. Biol. Med. 178 : 551-556.

Cattaneo, R., A. Schmid, M. A. Billeter, R. D. Sheppard and S. A. Udem. 1988. Multiple viral mutations rather than host factors cause defective measles virus gene expression in a subacute sclerosing panencephalitis cell line. J. Virol. 62 : 1388-1397. J. Virol. 62 : 1388-1397.

Choppin, P. W., C. D. Richardson, D. C. Mertz, W. W. Hall and A. Scheid. 1981. The functions and inhibition of the membrane glycoproteins of paramyxoviruses and myxoviruses and the role of the measles virus M protein in subacute sclerosing panencephalitis. J. Inf. Dis. 143 : 352-363.

Chui, L. W. L., R. Vainionpää, R. Marusyk, A. Salmi and E. Norrby. 1986. Nuclear accumulation of measles virus nucleoprotein associated with a temperature sensitive mutant. J. Gen. Virol. 67. 2153-2161.

Coovadia, H. M., A. Wesley and P. Brain. 1978. Immunological events in acute measles influencing outcome. Arch. Dis. Child. 53 : 861-867.

Coovadia, H. M., A. Wesley, L. G. Henderson, P. Brain, G. H. Vos, and A. F. Hallett. 1978. Alterations in immune responsiveness in acute measles and chronic post-measles chest disease. Int. Archs. Aller. Appl. Immun. 56 : 14-23.

Coovadia, H. M., A. Wesley, M. G. Hammond and P. Kiepiela. 1981. Measles, Histocompatibility leukocyte antigen polymorphism, and natural selection in humans. J. Inf. Dis. 144 : 142-147.

Crowley, J. C., P. T. Dowling, J. Mennona, B. Schanzer, E. D. Young, S. D. Cook and B. M. Blumberg. 1987. Molecular cloning of 99% of measles virus genome, positive identification of 5' end clones, and mapping of the L gene region. Intervirology. 28: 65-77.

Crowley, J. C., P. T. Dowling, J. Mennona, J. I. Silverman, D. Schuback, S. D. Cook and B. M. Blumberg. 1988. Sequence variability and function of measles virus 3' and 5' ends and intercistronic regions. Virology. 164 : 498-506.

De Maeyer, E., J. De Maeyer-guignard and M. Vandeputte. 1975. Inhibition by interferon of delayed-type hypersensitivity in the mouse. Proc. Natl. Acad. Sci. USA. 72 : 1753-1757.

Dowling, P. C., B. M. Blumberg, J. Mennowa, J. E. Adamus., P. Cook, J. C. Crowley, D. Kolakowsky, D. and S. T. Cook. 1986. Transcriptional map of the measles virus genome. J. Gen. Virol. 67 : 1987-1992.

Dubois-Dalcq, M., J. M. Coblentz and A. B. Pleet. 1974. Subacute sclerosing panencephalitis. Unusual nuclear inclusions and lenghty clinical course. Arch. Neurol. 31 : 355-363.

Dubois-Dalcq, M., K. W. Holmes and B. Rentier. 1984. "Assembly of Enveloped RNA viruses". pp. 44-64. Springer-Verlag, New York/Vienna.

Dunmire, C., J. C. Ruchdeschel and M. R. Mardiney, Jr. 1975. Suppression of in vitro lymphocyte responsiveness to purified protein derivative by measles virus. A reexploration of the phenomenon. Cell. Immunol. 20 : 205-211.

Enders, J. F. and T. C. Preebles. 1954. Propagation in tissue cultures of cytopathogenetic agents from patients with measles. Proc. Soc. Biol. Med. 86: 277-286.

Fluks, A. J. 1981. Three-step isolation of human monocytes using discontinuous density gradients of percoll. J. Immunol. Met. 41 : 225-233.

Fournier, J. G., M. Tardieu, P. Lebon, O. Robain, G. Ponsot, S. Rozenblatt and M. Bouteille. 1985. Detection of measles virus RNA in lymphocytes from peripheral blood and brain perivascular infiltrates of patients with subacute sclerosing panencephalitis. N. Eng. J. Med. 313 : 910-915.

Fournier, J. G., J. Gerfaux, A. M. Joret and P. Lebon. 1988. Subacute sclerosing panencephalitis: detection of measles virus sequences in RNA extracted from circulating lymphocytes. B. Med. J. 296 : 684.

Francois, D. T., I. M. Katona, C. H. June. L. M. Wahl and J. J. Mond. 1988. Examination of the inhibitory and stimulatory effects of IFN-α, -β, -γ on human B cell proliferation induced by various B-cell mitogens. Clin. Immunol. Immunopathol. 48: 297-306.

Fraser, K. B. and S. J. Martin. 1978. Measles virus and its biology. pp 41-54. Academic Press Inc., New York.

Fujinami, R. S., E. Norrby and M. B. A. Oldstone. 1984. Antigenic modulation induced by monoclonal antibodies: antibodies to measles virus hemagglutinin alters expression of other viral polypeptides in infected cells. J. Immunol. 132 : 2618-2621.

Fujinami, R. S. and M. B. A. Oldstone. 1981. Failure to cleave measles virus fusion protein in lymphoid cells. A possible mechanism for viral persistence in lymphocytes. J. Exp. Med. 154 : 1489-1499.

Giuffre, R. M., D. R. Tovell, C. M. Kay and D. L. J. Tyrrell. 1982. Evidence for an interaction between the membrane protein of a paramyxovirus and actin. J. Virol. 42 : 963-968.

Glickman, S., M. M. Ferm, W. Ou and J. A. Steitz. 1988. Structural analysis of EBER1 and EBER2 ribonucleoprotein particles present in Epstein-Barr virus-infected cells. J. Virol. 62 : 902-911.

Gould, E. A. and P. E. Linton. 1975. The production of a temperature sensitive persistent measles virus infection. J. Gen. Virol. 28 : 21-28.

Graves, M. C. 1981. Measles virus polypeptides in infected cells studied by immune precipitation and one dimensional peptide mapping. J. Virol. 38 : 224-230.

Graves, M. C., S. M. Silver and P. W. Choppin. 1978. Measles virus polypeptide synthesis in infected cells. Virology. 86 : 154-163.

Gray, M. M., I. M. Hann, S. Glass, O. B. Eden, P. M. Jones and R. F. Stevens. 1987. Mortality and morbidity caused by measles in children with malignant disease attending four major treatment centres: a retrospective review. Brit. Med. J. 295 : 19-24.

Graziano, K. D., J. C. Ruckdeschel and M. R. Mardiney, Jr. 1975. Cell associated immunity to measles (rubeola). The demonstration of in vitro lymphocyte tritiated thymidine incorporation in response to measles complement fixation antigen. Cell. Immunol. 15 : 347-354.

Gresser, I. 1977. On the varied biologic effects of interferon. Cell. Immunol. 34 : 406-415.

Griffith, D. E., T. R. Moench, R. T. Johnson, I. Lindo de Soriano and A. Vaisberg. 1986. Peripheral blood mononuclear cells during natural measles virus infection: Cell surface phenotype and evidence for activation. Clin. Immunol. Immunopathol. 40 : 305-312.

Griffith, D. E., R. T. Johnson, V. G. Tamashiro, T. E. Moench, E. Jauregui, I. Lindo de Soriano and A. Vaisberg. 1987. In vitro studies of the role of monocytes in the immunosuppression associated with natural measles virus infections. Clin. Immunol. Immunopathol. 45 : 375-383.

Hall, W. H. and S. J. Martin. 1973. Purification and characterisation of measles virus. J. Gen. Virol. 19 : 175-188.

Hall, W. H. and S. J. Martin. 1974. Structure and function relationship of the envelope of measles virus. Med. Microbiol. Immunol. 160 : 143-154.

Hall, W. W. and S. J. Martin. 1974. The biochemical and biological characteristics of the surface components of measles virus. J. Gen. Virol. 22 : 363-374.

Hall, W. W., W. Kiessling and V. ter Meulen. 1978. Membrane proteins of subacute sclerosing panencephalitis and measles virus. Nature. 272 : 460-462.

Hall, W. W., R. A. Lamb. and P. W. Choppin. 1979. Measles and subacute scleosing panencephalitis virus proteins: Lack of antibodies to the M proteins in patients with subacute sclerosing panencephalitis. Proc. Natl. Acad. Sci. USA. 76 : 2047-2051.

Hamilton, R., L. Barbosa and M. Dubois. 1973. Subacute sclerosing panencephalitis measles virus: Study of biological markers. J. Virol. 12 : 632-642.

Hanjan, S. N. S., J. F. Kearney and M. D. Cooper. 1982. A monoclonal antibody (MMA) that identifies a differentiation antigen on human myelomonocytic cells. Clin. Immunol. Immunopathol. 23 : 172-188.

Hardwick, J. M. and R. H. Bussell. 1978. Glycoproteins of measles virus under reducing and nonreducing conditions. J. Virol. 25 : 687-692.

Hasel, K. W., S. Day, S. Millward, C. D. Richardson, W. J. Bellini and P. T. Greer. 1987. Characterisation of cloned measles virus mRNA by in vitro transcription, translation and immunoprecipitation. Intervirology. 28 : 26-39.

Haspel, M. V., R. Duff and F. Rapp. 1975. Isolation and preliminary characterisation of temperature sensitive mutants of measles virus. J. Virol. 16 : 1000-1009.

Haspel, M. V., P. R. Knight, R. G. Duff and F. Rapp. 1973. Activation of a latent measles virus infection in Hamster cells. J. Virol. 12 : 690-695.

Hektoen, L. 1905. Experimental measles. J. Inf. Dis. 2 : 238-255.

Hirsch, R. L., D. E. Griffih, R. T. Johnson, S. J. Cooper, I. Lindo de Soriano, S. Roedenbeck and A. Vaisberg. 1984. Cellular immune response during complicated and uncomplicated measles virus infections of man. Clin. Immunol. Immunopathol. 31 : 1-12.

Holland, J. J., S. I. T. Kennedy, B. L. Semler, C. L. Jones, L. Roux and E. A. Grabau. 1980. Defective interfering particles RNA viruses and the host cell response, In Comprehensive Virology, (eds. Fraenkel-Conrat and R. R. Wagner) 16 : 137-183.

Holland, J. J. Spindler, F. Horodyski, E. Grabau, S. Nichol and S. Vandepol. 1982. Rapid evolution of RNA genomes. Science. 215 : 1577-1585.

Howe, C. 1979. Measles virus proteins and receptors. In Receptors and human diseases. pp 63-79 (eds. A. G. Bearn and P. W. Choppin), Josiah Nacy Jr. Foundation, New York.

Huddlestone, J. R., P. W. Lambert and M. B. A. Oldstone. 1980. Virus-lymphocyte interactions : Infection of T_G and T_M subsets by measles virus. Clin. Immunol. Immunopathol. 15 : 502-509.

Hyypiä, T., P. Korkiamäki and R. Vainionpää. 1985. Replication of measles virus in human lymphocytes. J. Exp. Med. 161 : 1261-1271.

Ilonen, J., R. Salonen, R. Marusyk and A. Salmi. 1988. Measles virus strain-dependent variation in outcome of infection of human peripheral blood mononuclear cells. J. Gen. Virol. 69 : 247-252.

Itoh, M., H. Shibuta and M. Homma. 1987. Single amino acid substitution of Sendai virus at the cleavage site of the fusion protein confers trypsin resistance. J. Gen. Virol. 68 : 2939-2944.

Jacobson, S., and H. McFarland. 1982. Measles virus persistence in human lymphocytes: A role for virus induced interferon. J. Gen. Virol. 63 : 351-357.

Joffe, M.I., N. R. Sukha and A. R. Robson. 1983. Lymphocytes subsets in measles. Depressed helper/inducer subpopulations reversed by in vitro treatment with levamisole and ascorbic acid. J. Clin. Invest. 72 : 971-977.

Johnson, H. M., G. S. Bennett and S. Baron. 1975. Inhibition of the primary in vitro antibody response by interferon preparations. J. Immunol. 114 : 403-409.

Joklik, W. K. 1985. Interferons. In Virology pp.281-307 (eds. B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman and R. E. Shope), Raven Press, New York.

Joseph, B. S., P. W. Lampert and M. B. A. Oldstone. 1975. Replication and persistence of measles virus in defined subpopulations of human leukocytes. J. Virol. 16 : 1638-1649.

Joseph, B. S. and M. B. A. Oldstone. 1975. Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. J. Exp. Med. 412 : 864-876.

Ju, G., M. Birrer, S. Udem and B. R. Bloom. 1980. Complementation analysis of measles virus mutants isolated from persistently infected lymphoblastoid cell lines. J. Virol. 33 : 1004-1012.

Ju, G., S. Udem, B. Rager-Zisman and B. R. Bloom. 1978. Isolation of a heterogeneous population of temperature sensitive mutants of measles virus from persistently infected human lymphoblastoid cell lines. J. Exp. Med. 147 : 1637-1652.

Julius, M. S., E. Simpson and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3 : 645-649.

Katz, M. 1974. Measles and central nervous system disease. A critical appraisal. Med. Microbiol. Immunol. 160 : 247-250.

Katz, S. L. 1982. Measles. In. Pediatrics pp 621-625 (eds. H. L. Barnett and A. H. Einhorn) Appleton-Century-Crofts, New-York.

Kempe, C. H. and Fulginiti, V. A. 1965. The pathogenesis of MV infection. Arch. Ges. Virusforsch. 16 : 103-128.

Kiley, M. P., R. H. Gray and F. E Payne. 1974. Replication of measles virus: Distinct species of short nucleocapsids in cytoplasmic extracts of infected cells. J. Virol. 13 : 721-728.

Kimura, Y., Y. Ito, K. Shimokata, Y. Nishiyama, I. Nagata and J. Kitoh. 1975. Temperature sensitive virus derived from BHK cells persistently infected with HVJ (Sendai Virus). J. Virol. 15 : 55-63.

Kingsbury, D. W. 1985. Orthomyxo- and paramyxoviruses and their replication. In Virology pp. 1179-1239 (eds. B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman and R. E. Shope), Raven Press, New York.

Kingsbury, D. W., M. A. Bratt, P. W. Choppin, R. B. Hanson, Y. Horaka, E. Norrby, W. Plowright, R. Rott and W. Wunner. 1978. Paramyxoviridae. Intervirology. 10 : 137-152.

Kipps, A. O., J. W. Moodie, D. W. Beatty, E. B. Dowdle and J. P. Mcintyre. 1982. Increased susceptibility to herpes simplex virus infections in children with acute measles. Infect. Immun. 31 : 1-6.

Knight, P., R. Duff and F. Rapp. 1972. Latency of human measles virus in hamster cells. J. Virol. 10 : 995-1001.

Kohama, T., T. A. Sato, F. Kohune and A. Sugiura. 1985. Maturation of measles virus hemagglutinin glycoprotein. Arch. Virol. 85 : 257-268.

Kohama, T., A. Fukuda and A. Sugiura. Effect of carboxylic ionophores on measles virus hemagglutinin protein. 1986. Arch. Virol. 89 : 213-223.

Krah, D. L. and P. W. Choppin. 1988. Mice immunised with measles virus develop antibodies to a cell surface receptor for binding virus. J. Virol. 62 : 1565-1572.

Krilov, L. R., R. M. Henry, E. Godfrey and K. McIntosh. 1987. Respiratory virus infection of peripheral blood monocytes: Correlation with ageing of cells and interferon production in vitro. J. Gen. Virol. 68 : 1749-1753.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 277 : 680-685.

Lebon, P., G. Ponsot, J. Aicardi, F. Goutieres and M. Arthuis. 1979. Early intarthecal synthesis of interferon in Herpes encephalitis. Biomed. 31 : 267-271.

Linnavuori, K. and T. Hovi. 1981. Herpes simplex virus infection in human monocyte cultures: dose dependent inhibition of monocyte differentiation resulting in abortive infection. J. Gen. Virol. 52 : 381-385.

Lucas, A., M. Coulter, R. Anderson, S. Dales and W. Flintoff. 1978. In vivo and in vitro models of demyelinating diseases. Virology. 88 : 325-337.

Lucas, C. J., J. M. D. Galama and J. C. Ubels-postman. 1978. Measles virus-induced suppression of lymphocyte reactivity in vitro. Cell. Immunol. 32 : 70-85.

Lucas, C. J., J. C. Ubels-postman, A. Rezee and J. M. D. Galama. 1978. Activation of measles virus from silently infected human lymphocytes. J. Exp. Med. 148 : 940-952.

Lund, G. A. and A. Salmi. 1981. Purification and characterisation of measles virus haemagglutinin protein G. J. Gen. Virol. 56 : 185-193.

Lund, G., D. L. J. Tyrrell, R. D. Bradley and D. G. Scraba. 1984. The molecular length of measles virus RNA and the structural organisation of measles nucleocapsids. J. Gen. Virol. 65 : 1535-1542.

Maniatis, T., E. F. Fritsch and J. Sambrook. 1982. Extraction, purification and analysis of mRNA from eukaryotic cells. In Molecular Cloning. A laboratory manual. pp 188-209. Cold Spring Harbour, New York.

Markowitz, L. E. and R. H. Bernier. 1987. Immunization of young infants with Edmonston-Zagreb measles vaccine. Pediatr. Infect. Dis. J. 6 : 809-812.

Martin, J. P., F. Koehren, J. J. Rannou and A Kirn. 1988. Temperature sensitive mutants of mouse hepatitis virus type 3(MHV-3): isolation, biochemical and genetic characterisation. Arch. Virol. 100 : 147-160.

Marusyk, R. G. 1984. Measles: A continuing problem or eradication. In Applied Virology pp 211 : 219 (ed. E. Kurstak) Academic Press Inc.

Mathes, L. E, R. G. Olsen, L. C. Hebebrand, E. A. Hoover, J. P. Schaller, P. W. Adams and W. S. Nichols. 1979. Immunosuppressive properties of a virion polypeptide, a 15,000-dalton protein from feline leukemia virus. Can. Res. 39 : 950-955.

Mayernick, D. G., A. Haq and J. J. Rinehart. 1984. Interleukin 1 secretion by human monocytes and macrophages. J. Leuk. Biol. 36 : 551-557.

McChesney, M. B., J. H. Kehrl, A. Valsamakis, A. S. Fauci, and M. B. A. Oldstone. 1987. Measles virus infection of B lymphocytes permits cellular activation but blocks progression through the cell cycle. J. Virol. 61 : 3441-3447.

Mckay, E., P. Higgins, D. Tyrrell and C. Pringle. 1988. Immunogenicity and pathogenicity of temperature sensitive modified respiratory syncytial virus in adult volunteers. J. Med. Virol. 25 : 411-421.

Mims, C. A. 1978. General features of persistent virus infections. Postgrad. Med. J. 54 : 581-586.

Mims, C. A. 1986. Inetractions of viruses with the immune system. Clin. Exp. Immunol. 66 : 1-16.

Minagawa, T., C. Nakaya and H. Iida. 1974. Host DNA synthesis-suppressing factor in culture fluid of tissue cultures infected with measles virus. J. Virol. 13 : 118-1125.

Minagawa, T., T. Sakuma, S. Kuwajima, T. K. Yamamoto and H. Iida. 1976. Characterisation of measles viruses in establishment of persistent infections in human lymphoid cell line. J. Gen. Virol. 33 : 361-379.

Moench, T. R., D. E. Griffirh, R. Obriecht, A. B. Vaisberg and R. T. Johnson. 1988. Acute measles in patients with or without neurological involvement : distribution of measles virus antigen and RNA. J. Inf. Dis. 158 : 433-442.

Morgan, E. M. and F. Rapp. 1977. Measles virus and its associated diseases. Bact. Rev. 41 : 636-666.

Mountcastle, W. E. and P. W. Choppin. 1977. A comparison of the polypeptides of four measles virus strains. Virology. 78 : 463-474.

Nakai, T., F. L. Shand and A. F. Howatson. 1969. Development of measles virus in vitro. Virology 38 : 50-67.

Narayan, O. S. Kennedy-Stoskopf, D. Sheffer, D. E. Griffin and J. E. Clements. 1983. Activation of Caprine arthritis-encephalitis virus expression during maturation of monocytes to macrophages. Infect. Immun. 41 : 67-73.

Neighbour, P. A. and B. R. Bloom. 1979. Absence of virus induced lymphocyte suppression and interferon production in multiple Sclerosis. Proc. Natl. Acad. Sci. USA. 76: 476-480.

Norrby, E. 1967. A carrier cell line of measles virus in Lu 106 cells. Arch. ges. Virusforsch. 17 : 436-442.

Norrby, E. 1985. Measles. In Virology pp. 1305-1330 (eds. B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman and R. E. Shope), Raven Press, New York.

Norrby, E., H. Marusyk and C. Orvell. 1970. Ultrastructural studies of the multiplication of RS (respiratory syncytial) virus. Acta Pathol. Microbiol. Scand.(B) 78 : 268.

Oldstone, M. B. A. 1984. Viruses can alter cell function without altering cell pathology: Disordered functions leads to imbalance of homeostasis and disease. In. Concepts in Viral Pathogenesis. pp. 269-276. (eds. Notkins, A. L. and M. B. A. Oldstone). Springer-Verlag. New-York.

Oldstone, M. B. A. 1986. Distortion of cell functions by noncytotoxic viruses. Host. Prac. 15 : 82-92.

Oldstone, M. B. A. and R.S. Fujinami. 1982. Virus persistence and avoidance of immune surveillance: How measles viruses can be induced to persist in cells, escape immune assault and injure tissues. In Virus persistence. pp. 185-202. ((eds. B. W. J. Mahy, A. C. Minson and G. K. Darby) Cambridge University Press. London.

Orren, A., A. Kipps, J. W. Moodie, D. W. Beatty, E. B. Dowdle and Janet P. McIntyre. 1981. Increased susceptibility to herpes simplex virus infections in children with acute measles. Infect. Immun. 31 : 1-6.

Osler, W. 1892. The principles and practice of medicine: pp. 77. New York: D. Appleton and Co.

Osunkoya, B. O., A. R. Cooke, O. Ayeni, and T. A. Adejumo. 1974a. Studies on leucocytes cultures in measles. I. Lymphocyte transformation and giant cell formation in leucocyte cultures from clinical cases of measles. Arch. Ges. Virusforsch. 44 : 313-322.

Osunkoya, B. O., G. I. Adeleye, T. A. Adejumo and L. S. Salimonu. 1974b. Studies on leucocytes cultures in measles. II. Detection of measles virus antugens in human leucocytes by immunofluorescence. Arch. Ges. Virusforsch. 44 : 323-329.

Perignon, J. L., D. M. Bories, A. M. Houllier, L. Thuillier and P. H. Cartier. 1987. Metabolism of pyrimidine bases and nucleosides by pyrimidine-nucleoside phosphorylases in cultured human lymphoid cells. Biochim. Biophys. Acta. 928 : 130-136.

Pitkäranta, A., K. Linnavuori, M. Roivainen and T. Hovi. 1988. Induction of interferonα in human leukocytes by polioviruses: Wild-Type strains are better inducers than attenuated strains. Virology. 165 : 476-481.

Preble, O. T. and J. S. Younger. 1973. Selection of temperature sensitive mutants during persistent infection.: Role in maintenance of persistent Newcastle disease virus infections of L cells. J. Virol. 12 : 481-491.

Rake, G. and M. F. Schaffer. 1939. Propagation of the agent of measles in the fertile hen's egg. Nature. 144 : 672-673.

Rake, G., M. F. Schaffer and H. P. Jones 1941. Studies on measles. II. The use of tissue culture in propagation of measles virus. J. Inf. Dis. 69 : 65-69.

Ramig, R. F. 1985. Principles of animal virus genetics. In Virology pp. 129-144 (eds. B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman and R. E. Shope), Raven Press, New York.

Ray, J. and R. S. Fujinami. 1987. Characterisation of in vitro transcription and transcriptional products of measles virus. J. Virol. 61 : 3381-3387.

Richardson, C. D., A. Berkovich, S. Rozenblatt and W. J. Bellini. 1985. Use of antibodies directed against synthetic peptides for identifying cDNA clones, establishing reading frames, and deducing the gene order of measles virus. J. Virol. 54 : 186-193.

Richardson, C., D. Hull, P. Greer, K. Hasel, A. Berkovich, G. Englund, W. Bellini, B. Rima and R. Lazzarini. 1986. The nucleotide sequence of the mRNA encoding the fusion protein of measles virus (Edmonston) strain: A comparison of fusion proteins from several different paramyxoviruses. Virology. 155 : 508-523.

Richman, D. D. and B. R. Murphy. 1979. The association of the temperature sensitive phenotypes with viral attenuation in animals and humans: Implications for the development and use of live virus vaccines. J. Inf. Dis. 1 : 413-433.

Rima, B. K. 1983. The proteins of morbilliviruses. J. Gen. Virol. 64 : 1205-1219.

Rima, B. K., W. B. Davidson and S. J. Martin. 1977. The role of defective interfering particles in persistent infection of Vero cells by measles virus. J. Gen. Virol. 35 : 89-97.

Rima, B. K. and S. J. Martin. 1976. persistent infection of tissue culture cells by RNA viruses. Med. Microb. Immunol. 162 : 89-118.

Rima, B. K. and S. J. Martin. 1979. Effect of undiluted passage on the polypeptides of measles virus. J. Gen. Virol. 44 : 135-144.

Rima, B. K., K. Baczko, D. K. Clarke, M. D. Curran, S. J. Martin, M. A. Billeter and V. ter Meulen. 1986. Characterisation of clones for the sixth (L) gene and a transcriptional map for morbillivirus. J. Gen. Virol. 67 : 1971-1978.

Rinehart, J. J., M. Orser and M. E. Kaplan. 1979. Human monocytes and macrophage modulation of lymphocyte proliferation. Cell. Immunol. 44 : 131-143.

Robbins, S. J. and R. H. Bussell. 1979. Structural phosphoproteins associated with purified measles virions and cytoplasmic nucleocapsids. Intervirology. 12 : 96-102.

Robbins, S. J., R. H. Bussell and F. Rapp. 1980. Isolation and characterisation of two forms of cytoplasmic nucleocapsids from measles virus-infected cells. J. Gen. Virol. 47 : 301-310.

Robertson, D. A. F., S. L. Zhang, E. C. Guy and R. Wright. 1987. Persistent measles virus genome in autoimmune chronic active hepatitis. Lancet 1 : 9-11.

Roitt, I., J. Brostoff and D. Male. 1985. Immunology. pp 11.7-11.8. Gower medical publishing. C. V. Mosby Company. St. Louis. Missouri.

Roizman, B. 1985. Multiplication of viruses: an overview. in Virology pp. 69-76 (eds. B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman and R. E. Shope), Raven Press, New York.

Rustigian, R. 1966a. Persistent infection of cells in culture by measles virus. I. Development and characteristics of HeLa Sublines persistently infected with complete virus. J. Bact. 92 : 1792-1804.

Rustigian, R. 1966b. Persistent infection of cells in culture by measles virus. II. Effect of measles antibody on persistently infected Hela sublines and recovery of a Hela clonal line persistently infected with incomplete virus. J. Bact. 92 : 1805-1811.

Sanchez-Lanier, M., P. Guerin, L. C. Mclaren, and A. D. Bankhurst. 1988. Measles virus-induced suppression of lymphocyte proliferation. Cell. Immunol. 116 : 367-381.

Saksela, E., I. Virtanen, T. Hovi, D. S. Secher and K. Cantell. 1984. Monocyte is the main proucer of human leukocyte alpha interferons following Sendai virus induction. Prog. Med. Virol. 30 : 78-86.

Salonen, R., J. Ilonen, M. Reunanen, J. Nikoskelainen and A. Salmi. 1982. PPD-, PWM-, and PHA-induced interferon in stable multiple sclerosis: Association to HLA-Dw2 antigen and clinical parameters. Ann. Neurol. 11 : 279-284.

Salonen, R., J. Ilonen and A. Salmi. 1988. Measles virus infection of unstimulated blood mononuclear cells in vitro: antigen expression and virus production preferentially in monocytes. Clin. Exp. Immunol. 71 : 224-228.

Salonen, R., J. Ilonen and A. Salmi. 1989. Measles virus inhibits lymphocyte proliferation in vitro by two different mechanisms. Clin. Exp. Immunol. 75 : 376-380.

Sandford, G,. R. and W. H. Burns. 1988. Use of temperature sensitive mutants of mouse cytomegalovirus as vaccines. J. Inf. Dis. 158 : 596-601.

Sato, T. A., T. Kohama and A. Sagiura. 1988. Intracellular processing of measles virus fusion protein. Arch. Virol. 98 : 39-50.

Schattner, A., A. Meshorer and D. Wallach. 1983. Involvement of interferon in virus induced lymphopenia. Cell. Immunol. 79 : 11-25.

Schmidt, N. J. 1979. Cell culture techniques for diagnostic virology. in Diagnostics procedures for viral rickettsial and chlamidial infections. (eds. E, H, Lennette and N. J. Schmidt). American Public Health Association.

Scott, J,. E. and P. W. Choppin. 1982. Enhanced yields of measles virus from cultured cells. J. Virol. Met. 5 : 173-179.

Seifried, A. S., P. Albretch and J. B. Milstein. 1978. Characterisation of an RNA dependent RNA polymerase activity associated with measles virus. J. Virol. 30 : 166-176.

Shesberadaran, H., E. Norrby and K. W. Rammohan. 1985. Monoclonal antibodies against five structural components of measles virus. II. Characterisation of five persistently infected with measles virus. Arch. Virol. 83 : 251-268.

Simpanen, E., R. Von Essen and H. Isomäki. 1977. Remission of juvenile rheumatoid arthritis (Still's disease) after measles. Lancet ii: 987-988.

Sonnenfeld, G., A. D. Mandel and T. C. Merigan. 1977. The immunosuppressive effect of Type II mouse interferon preparations on antibody production. Cell. Immnol. 34 : 193-206.

Spruance, S. L., B. N. Ashton and C. B. Smith. 1980. Preparation and characterisation of high specific activity radiolabelled 50S measles virus RNA. J. Virol. Met. 1 : 223-223.

Stallcup, K. C., S. Wechsler and B. N. Fields. 1979. Purification of measles virus and characterisation of subviral components. 30 : 166-176.

Starr, S. and S. Berkovitch. 1964. Effect of measles, gamma globulin modified measles and vaccine measles on the tuberculin test. N. Engl. J. Med. 270 : 386-391.

St. Geme. J. W. 1964. Evidence for the nucleic acid composition of measles virus. Pediatrics. 71-74.

Sullivan, J. L., D. W. Barry, P. Albretch, and S. J. Lucas 1975. Inhibition of lymphocyte stimulation by measles virus. J. Immunol. 114 : 1458-1461

Synderman, R. and G. J. Cianciolo. 1984. Immunosuppressive activity of the retroviral envelope protein p15E and its possible relationship to neoplasia. Immunol. Today 5 : 240-242.

ter Meulen, V. and M. J. Carter. 1982. Morbillivirus persistent infections in animals and man. pp. 97-132. In Virus persistence (eds. B. W. J. Mahy, A. C. Minson and G. K. Darby) Cambridge University Press. London.

ter Meulen, V., M. Katz and Y. M. Käckell. 1973. Properties of SSPE virus; tissue culture and animal studies. Ann. Clin. Res. 5 : 293-297.

ter Meulen, V. 1974. Pathogenetic aspects of measles virus infection. Med. Microbiol. Immunol. 160 : 165-172.

Trinchieri, G. and B. Perussia. 1985. Immune interferon: a pleiotropic lymphokine with multiple effects. Immunol. Today. 6 : 131-137.

Tsang, L. W. L., N. Chang and R. G. Marusyk. 1981. An interference phenomenon associated with a measles virus SSPE isolate (Halle). J. Gen. Virol. 56: 195-198.

Tyrrell, D. L. J. and A. Ehrnst. 1979. Transmembrane communication in cells chronically infected with measles virus. J. Cell Biol. 81: 396-402.

Tyrrell, D. L. J. and E. Norrby. 1978. Structural proteins of measles virus. J. Gen. Virol. 39 : 219-229.

Tyrrell, D. L. J., D. J. Rafter, C. Orvell and E. Norrby. 1980. Isolation and immunological characterisation of the nucleocapsid and membrane proteins of measles virus. J. Gen. Virol. 51 : 307-315.

Udem, S. and K. A. Cook. 1984. Isolation and characterisation of measles virus intracellular nucleocapsid RNA. J. Virol. 49 : 57-65.

Vainionpää, R. 1979. Measles virus specified polypeptides in infected cells. Arch. Virol. 60 : 239-248.

Vainionpää, R., B. Ziola and A. Salmi. 1978. Measles virus polypeptides in purified virions and in infected cells. Acta. path. microbiol. scand. 86 : 379-385.

Volckaert-Vervliet, G., H. Heremans, M. De Ley and A. Billiau. 1978. Interferon induction and action in human lymphoblastoid cells infected with measles virus. J. Gen. Virol. 41; 451-466.

von Pirquet, C. P. 1908. Das Verhalten der kutanen Tuberculin reaktion während der masern. Dtsch. Med. Wochenschr. 34 : 1297-1300.

Wainberg, M. A., S. Vydelingum and R. G. Margolese. 1983. Viral inhibition of lymphocyte mitogenesis : interference with the synthesis of functionally active T cell growth factor and reversal of inhibition by addition of same. J. Immunol. 130 : 2372-2378.

Wainberg, M. A., S. Vydelingum, J. Boushira, J. Legace-Simard, R. G. Margolese, B. Spira and J. Mendelson. 1984. Reversible interference with TCGF activity by virus particles. Clin. Exp. Immnol. 57 : 663-670.

Walsh, J. A. 1983. Selective primary health care : strategies for control of disease in the developing world. IV. Measles. Rev. Inf. Dis. 5 : 330-340.

Waterson, A. P. 1965. Measles virus. Arch. Ges. Virusforsch. 16 : 103-128.

Waterson, A. P., J. G. Cruickshank, G. D. Lawrence and A. D. Kanarek. 1961. The nature of measles virus. Virology. 15 : 379-382.

Wechler, S. L. and B. N. Fields. 1978. Difference between the intracellular polypeptides of measles and subacute sclerosing panencephalitis virus. Nature. 272 : 458-460.

Wechsler, S. L., D. M. Lambert, M. S. Galinski, M. A. Mink, O. Rochovansky and M. W. Pons. 1987. Immediate persistent infection by human parainfluenzae virus 3: Unique fusion properties of the persistently infected cells. J. Gen. Virol. 68: 1737-1748.

Wesley, A., H. M. Coovadia and L. Henderson. 1978. Immunological recovery after measles. Clin. Exp. Immunol. 32: 540-544.

White, D. O. and F. Fenner. 1987. Viral genetics and evolution. in Medical Virology pp. 91-118. Academic Press Inc. (London) LTD.

White, J., M. Kielian and A. Helenius. 1983. Membrane fusion proteins of enveloped animal viruses. Quart. Rev. Biophys. 16 : 151-195.

White, R. G. and J. F. Boyd. 1973. The effect of measles on the thymus and other lymphoid tissues. Clin. Exp. Immunol. 13 : 343-357.

Whittle, H. C., J. Dossetor, A. Oduloju and A. D. M. Bryceson and B. M. Greenwood. 1978. Cell-mediated immunity during natural measles infection. J. Clin. Invest. 62 : 678-684.

Wild, T. F. and R. Dugre. 1978. Establishment and characterisation of a subacute sclerosing panencephalitis (measles) virus persistent infection in BGM cells. J. Gen. Virol. 39 : 113-124.

Wild, F. and T. Greenland. 1979. A study of the measles virus-induced proteins incorporated into the cell membrane. Intervirology. 11 : 275-281.

Wiley, D. C. 1985. Viral membranes. In Virology pp. 45-67. (eds. B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman and R. E. Shope), Raven Press, New York.

William, P. J. and H. F. Hull. 1983. Status of measles in the Gambia, 1981. Rev. Infect. Dis. 5 : 391-394.

Wilson, G. S. 1962. Measles as a universal disease. Am. J. Dis. Child. 103 : 49-53.

Winston, S. H., R. Rustigian and M. A. Bratt. 1973. Persistent infection of cells in culture by measles virus. III. Comparison of virus specific RNA synthesized in primary and persistent infection in Hela cells. J. Virol. 11 : 926-932.

Wong, T. and A. Hirano. 1987. Structure and function of bicistronic RNA encoding the phosphoprotein and matrix proteins of measles virus. J. Virol. 61 : 584-589.

Yoshikawa, Y., K. Mizumoto and K. Yamanouchi . 1986. Characterisation of messenger RNAs of measles virus. J. Gen. Virol. 67 : 2807-2812.

Yoshioka, K., H. Miyata and S. Maki. 1981. Transient remission of juvenile rheumatoid arthritis after measles. Acta. Paediatr. Scand. 70 : 419-420.

Zweiman, B. 1971. In vitro effects of measles virus on proliferating human lymphocytes. J. Immunol. 106 : 1154-1158.